

THE LIVER IN DISEASE:
with special reference to Aspiration
Liver Biopsy.

- by -

SHEILA PATRICIA VIOLET SHERLOCK.

M.D.

1945

Thesis submitted to the Faculty of Medicine of
the University of Edinburgh for the degree of
Doctor of Medicine.

September, 1945.



THE LIVER IN DISEASE.

Part I.

TABLE OF CONTENTS.

	Page
Chapter 1. Introduction.	5
Chapter 2. The technique of aspiration liver biopsy.	8
Chapter 3. Biochemical methods in liver diseases: a critical study.	
Introduction and methods.	19
Chapter 4. Serum bilirubin estimations.	28
Chapter 5. Serum alkaline phosphatase estimations.	38
Chapter 6. Serum cholesterol estimations	47
Chapter 7. Total and differential serum protein estimations.	53
Chapter 8. Galactose tolerance and the liver.	61
Chapter 9. Hippuric acid synthesis.	76
Chapter 10. Biochemical methods in liver diseases. Discussion and con- clusions.	105
Chapter 11. Acute hepatitis. Introduction.	
General pathology.	112
Chapter 12. The course of acute hepatitis. Fatal hepatitis. The mode of recovery	129

	Page
Chapter 13. Post-hepatitis cirrhosis.	153
Chapter 14. The "post-hepatitis syndrome".	170
Chapter 15. Acute hepatitis. Summary of Chapters 11 to 14.	182

Chapter I.

INTRODUCTION.

The art of medicine is being ever expanded by newer techniques and ways of examination. Before any method can take its place for general use, its scope and applicability have first to be put on a firm basis. Aspiration liver biopsy is such a method. Although the use of liver puncture for the diagnosis of liver diseases was first reported over a hundred years ago (Roberts, 1833; Bielt, 1833), and the use of the method for the localisation of abscesses has been long used in the tropics, the method has never come into general use as a part of Western medicine. The fear of dangerous complications has been the main factor in preventing its wider use. In 1939 came the first real evidence that liver puncture could be without risk. In Denmark, Iversen and Roholm, using a simple procedure, reported 160 hepatic punctures with no mishaps. The coming of war, with its concomitant epidemics of hepatitis, made more accurate knowledge of the associated hepatic pathology essential. It was thought that aspiration liver biopsy might be the means of studying the histology of this condition.

This hope was realised, but early experiences suggested that the method was not entirely safe. In our first 126 punctures there were two deaths from intraperitoneal bleeding (Dible, McMichael and Sherlock, 1943). Since then a further 152 punctures have been performed with no evidence of haemorrhage. The change in technique which has so increased the safety will be discussed.

The technique being safe, it was necessary to determine the scope of the procedure. Many varieties of disease processes involving the liver have been studied. The method is of particular value in the investigation of diseases which are not usually fatal. Acute (epidemic) hepatitis and infectious mononucleosis are two particular conditions in which postmortem material is not readily available, and in which aspiration biopsy has enabled studies of the hepatic changes to be made. Moreover, the tissue obtained by aspiration biopsy technique can be fixed immediately, and is free from the autolytic changes so often observed in autopsy material. Serial biopsies can be performed and the changes in the liver during the course of the disease noted. In addition, the purely diagnostic value of the technique will be described with reference to a wide variety of diseases.

If used only for the diagnosis of the obscure case, aspiration liver biopsy will hold a place in clinical medicine.

This thesis commences with a detailed description of the technique and difficulties arising in its application. Then follows a comparison of the results of certain commonly employed biochemical methods used in the study of liver diseases with the histological appearance of the liver. The liver in acute hepatitis and obstructive jaundice is described in some detail, and next come reports of various cases in which the method has been applied either diagnostically or for the general study of the disease process. The thesis concludes with some possible applications of the method to the assessment of drug toxicity and the efficacy of therapeutic agents.

Chapter II.

THE TECHNIQUE OF ASPIRATION LIVER BIOPSY.

Despite the battery of laboratory tests available to assist in the study of hepatic diseases, definite diagnosis is often difficult. Any safe sure diagnostic method is therefore to be welcomed. When Iversen and Roholm (1939) published their series of aspiration liver biopsies, 160 biopsies with no mishaps, it was believed that this method might become generally applicable. However, early results obtained in this School (Dible, McMichael and Sherlock, 1943) indicated that the method was not without risk. Modifications in the selection of cases and in technique have now been made which have increased the safety of the procedure. The modified technique, its difficulties and risks will be described.

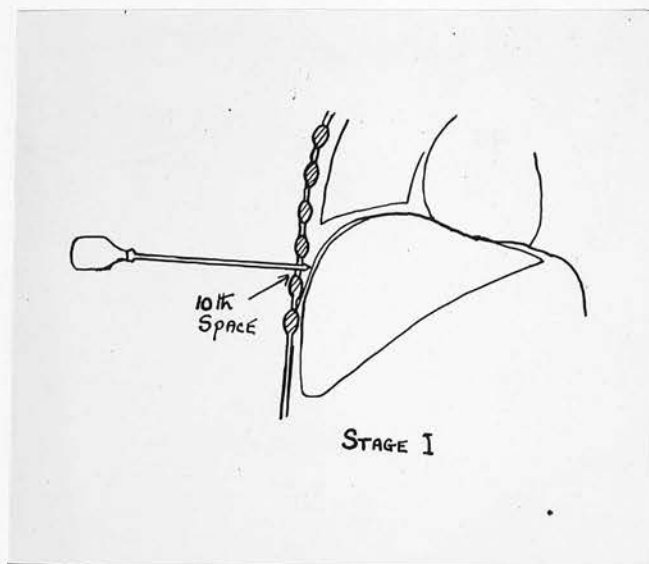
Preparation of the patient. In the jaundiced subject it is advisable to give a vitamin K preparation for three days before the puncture. If the jaundice is non-obstructive (urobilin present in the urine), then two 5 mg. tablets of Kapilon are given by mouth three times a day. If the jaundice is obstructive, then the vitamin must be administered intramuscularly: 5 mg. daily is a suitable dose.

In every case, before biopsy is performed, the patient's blood group should be known and two pints of compatible blood should be readily available.

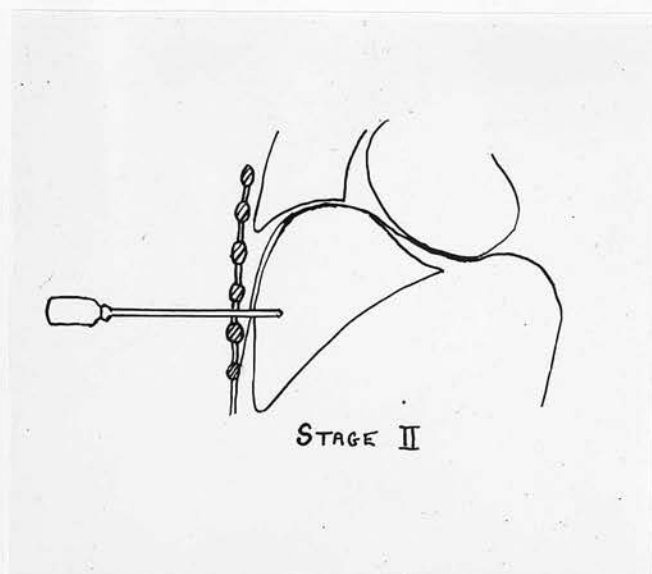
Aspiration liver biopsy should not be performed unless adequate facilities for blood transfusion are available in case of complicating haemorrhage. A sedative is unnecessary before puncture.

The hepatic puncture. The patient is lying supine in bed with the right side as near the edge of the bed as possible. A firm pillow may be placed under the left side in the hollow of the bed so that the body is slightly tilted to the right. The right arm is placed behind the head. A site is chosen in the ninth or tenth intercostal space in the middle or anterior axillary line. After cleansing, the skin is anaesthetised with 2 per cent novocaine solution and a long fine bore needle used to infiltrate the pleura; this is then passed through the diaphragm to anaesthetise the peritoneum and capsule of the liver. At least 10 ml. of local anaesthetic are needed. If the skin is tough, a preliminary nick may be made with a scalpel. The cannula used is 15 cm. long and 1 mm. in diameter. It is fitted with a handled trocar.* The instrument is passed through the skin (Fig. 1) and the patient then instructed to "take a deep breath in, let it out, and then hold your breath". This displaces the lung upwards and ensures apposition of diaphragmatic and costal pleura.

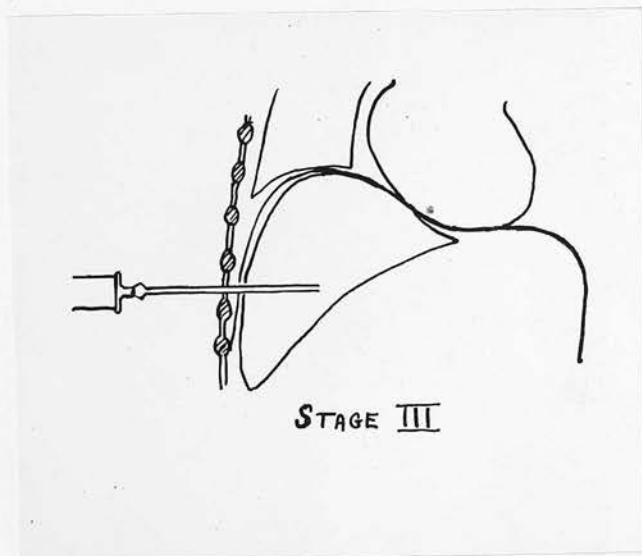
* The trocar and cannula were supplied by Down Bros., London.



2



3



Technique of aspiration

liver biopsy. stage I.

After preliminary local anaesthesia down to and including the capsule of the liver the trocar and cannula are inserted into the 9th or 10th intercostal space through the skin and intercostal muscles.

stage II.

With the subject holding his breath in expiration the trocar and cannula are passed through the diaphragm and into the liver substance.

stage III.

The trocar is withdrawn and the cannula pushed on a further 3-5 cms. Suction is applied with a 20 ml. syringe and the whole is withdrawn while suction is maintained.

The trocar and cannula are now pushed through the diaphragm into the right lobe of the liver. (Fig.2). The trocar is not withdrawn until the instrument is fully half an inch inside the liver substance. The cylinder of liver tissue is then punched out by pushing the cannula on a further 4-5 cm. (Fig. 3). A 20 ml. record syringe is connected to the cannula and suction is applied and maintained while the cannula is withdrawn. The puncture wound in the skin is sealed with collodion. The fragment of liver is usually found in the barrel of the syringe; occasionally it remains in the cannula. The aspiration of blood with the biopsy need not occasion alarm.

After care. As a little local pain may follow puncture, morphia gr. $\frac{1}{4}$ or gr. $\frac{1}{6}$ is given subcutaneously, according to the size and type of patient. This allays discomfort and prevents restlessness. If necessary, a further sedative such as gr.10 barbitone soluble may be given in the evening. An hourly pulse chart is kept for the first twenty-four hours after biopsy; the physician should be called if the pulse rate shows a rise. Routine visits should be paid 4 and 8 hours after biopsy. A very careful watch must be kept on the patient, and if there is any sign of haemorrhage the cross-matched blood should be administered.

Absolute bed rest is essential for twenty-four hours. The subject can then be up and about and if desired can leave hospital forty-eight hours after the liver puncture. The procedure is attended with very little disturbance to the patient. During the puncture there may be a complaint of a drawing feeling across the epigastrium. Afterwards some patients have a slight ache in the right side for about twenty-four hours and some complain of pain referred from the diaphragm to the right shoulder. Most patients agree that the discomfort compares favourably with that associated with sternal or lumbar puncture. 32 patients had more than one biopsy; one had four.

Difficulties in aspiration liver biopsy. There may be failure to get an adequate sample of liver. Hoffbauer (1944) (quoted Watson, 1944) had 40 per cent failures with the Tripoli and Fader technique. Iversen and Roholm (1939) reported a 10-15 per cent failure rate; Van Beek and Haex (1943), however, using the same method, state that the puncture failed but rarely. In our first 126 biopsies there were 10 per cent of failures; in the next 152 only 2 per cent.

Difficulties occur most frequently in cases of hepatic cirrhosis, especially if there is associated ascites. In cirrhosis the tough liver is difficult to pierce and a few liver cells may be extracted leaving the

fibrous framework behind. In ascites the liver is very "ballottable" and is difficult to transfix. A paracentesis abdominis should be undertaken before the liver biopsy is attempted and the patient should lie well over on the right side during the puncture. This brings the liver into contact with the chest wall. Another source of difficulty may be pulmonary emphysema; the liver is pushed downwards by the low diaphragm. It is very easy for the trocar to pass above the liver. If a low diaphragm is suspected before biopsy, reference should be made to a chest x-ray and if necessary the puncture can be made through a lower intercostal space.

TABLE I.

THE MORTALITY OF ASPIRATION LIVER BIOPSY

Author	Date	Number of Liver Biopsies	Deaths
Bingel	1923	100	2
Olivet	1926	140	3
Wardenström	1928	35	0
Huard, May & Joyeux	1935	163	0
Baron	1939	49	1
Iversen & Roholm	1939	160	0
Tripoli & Fader	1941	14	0
Hatieganu, Sparchez, Radu & Macavei	1943	45	0
Stahel	1943	70	0
Van Beek & Haex	1943	200	0
Hoffbauer	1945	65	0
Present series	1945	278	2
Totals		1319	8

Risks of aspiration liver biopsy. The fatality rate in published cases is shown in Table I. The percentage mortality is 0.61. In the first 126 punctures in this series (Dible et al., 1943) there were two deaths, one of which occurred in a patient already moribund and suffering from subacute liver necrosis. Since then a further 152 punctures have been performed

with no evidence of haemorrhage. This lessening of risk may be attributed to changes in technique and to more careful selection of cases.

The trocar used in the original series of 126 punctures was 2 mm. in diameter and 10 cm. long. As we have described, the new instrument is longer and of narrower bore. In one of the fatal cases the blood leakage had taken place from a cylindrical hole on the liver surface. The use of the narrower bored cannula diminishes the wound in the liver; the longer instrument allows the sharp trocar to be pushed well into the liver before it is withdrawn; the incision in the capsule is then clean cut and can heal easily, while the small cylinder of liver is taken deeply in the liver substance.

Accidents will be prevented if the biopsy is confined to patients who are cooperative. It is dangerous if the patient breathes with the trocar in the liver, as a longitudinal rent can then be produced. For this reason speed in puncture is essential. Any modification in technique, such as the injection of an anticoagulant through the trocar or the intercostal use of a more complicated needle such as that of Tripoli and Fader, will add to the time taken for the biopsy, and hence increase the risk of producing a rent in the liver. The risk of haemorrhage is greatest in the severely jaundiced, especially if the jaundice is due

to acute parenchymatous liver disease. We have never encountered clinical evidence of haemorrhage in the non-jaundiced group. Table II shows a comparison of the results in the previous series with those using the new technique in jaundiced patients.

TABLE II.

INCIDENCE OF HAEMORRHAGE AFTER ASPIRATION
LIVER BIOPSY IN JAUNDICED SUBJECTS.

Previous series (1943) Short 2mm.bore cannula		Present technique Long 1mm.bore cannula
Bleeding	5	0
No bleeding	78	76

Inspection and fixation of the liver biopsy. Some information may be gained by inspection of the biopsy. Fatty livers have a characteristic greasy look. Biopsies of livers with excess fibrous tissue tend to crumble into fragments which have a lobulated contour. If a malignant deposit has been punctured, the dull white appearance of the biopsy is characteristic.

The most useful routine fixative is absolute alcohol. Though it dissolves out red cells, this preserves the glycogen in the liver cells and enables Best's carmine stain to be applied. If it is desired to demonstrate the elements of blood in the hepatic

sinusoids, then formol saline is the more satisfactory fixative. Fat can be shown by fixing the material in Bouin's solution followed by 2 per cent osmic acid. After fixation the specimen is embedded, sectioned longitudinally along the line of the cylinder and stained in the usual way.

If desired, bacteriological examination of the sample can be carried out.

Use of aspiration liver biopsies as representative of liver pathology. The use of these small biopsies as representative of the pathology of the whole organ may be questioned. Pathologists, including Stewart (1917), Miller & Rutherford (1923), Bergstrand (1930) and Lucké (1944) recorded that in massive hepatic necrosis in man the left lobe of the liver is more damaged than the right. Himsworth & Glynn (1944) mention similar findings in experimental "trophopathic hepatitis". In the acute hepatitis cases the histological damage in our samples corresponded well with the clinical severity of the disease, and there was little variation from lobule to lobule. Excluding the obviously localised conditions such as malignant metastases, abscesses and cysts, most other examples of human liver disease have a reasonably uniform histology. Thirty cases came eventually to necropsy; in each instance the biopsy histology was proved to be a fair sample of the liver

as a whole. It must be emphasised that the preparation obtained from these biopsies is not a smear of liver cells but an actual section of liver tissue comprising about 10-20 lobules.

TABLE III.

THE DISTRIBUTION OF THE CASES STUDIED
BY ASPIRATION LIVER BIOPSY.

Diagnosis	Number of cases	Number of biopsies
Acute hepatitis:		
Epidemic hepatitis	23	29
Arsenotherapy jaundice	54	71
Serum jaundice	10	11
Cirrhosis of the liver	22	27
Obstructive jaundice	19	29
Malignant disease of the liver without jaundice	7	9
Diseases of the blood	22	22
Kala Azar	3	3
Amyloid disease	3	3
Cardiac failure	24	26
Miscellaneous	48	48
Total	235	278

The diagnostic application of aspiration liver biopsy.

The distribution of the case material is shown in Table III. The diagnostic potentialities of the method will

be discussed later in the thesis.

Summary.

The technique of aspiration liver biopsy is described. Difficulties and risks are mentioned. The use of the material as representative of liver pathology is discussed.

CHAPTER IIIA CRITICAL STUDY OF SOME BIOCHEMICAL INVESTIGATIONS
IN LIVER DISEASESIntroduction.

The use of biochemical techniques in the clinical investigation of liver diseases is indicated for two purposes: to arrive at an accurate diagnosis and to estimate the severity of the liver damage. The rationale for the various methods employed has hitherto been based on evidence provided by animal experiments or on records of cases of liver disease which have been verified by operation or necropsy findings. Assessment solely by clinical methods is often fallacious. An important approach to the evaluation of a liver function test is the comparison of results of biochemical tests with the histological appearance of the liver. The appearance of liver cells in section cannot of course be identified with the vital functions undertaken by these units. Changes in cell appearance do not represent a specific disturbance of function. However, until the establishment of a method directly estimating the workings of human liver cells "in vivo" or "in vitro", morbid histology would seem to be a reasonable index to the value of a liver function test. Each technique will be considered individually, both as an aid to diagnosis and in estimating the severity of the liver lesion. The results will then be discussed as a whole.

Methods.

Liver tissue has been obtained by a modification of the aspiration liver biopsy technique of Iversen and Roholm (1939). The method, its risks and applicability have been described in Chapter II. In a few instances

material obtained at necropsy or operation has been used. These latter sources may be unreliable.

Agonally glycogen usually disappears from the cells, and postmortem autolytic changes proceed rapidly (Van Beek and Haex, 1943). The necropsy may take place some time after the performance of the liver function test.

Material obtained by surgical exposure of the liver is subject to the effects of trauma and anaesthesia. Moreover, the interpretation of small samples from the extreme liver edge is often difficult. The aspiration liver biopsy can be done in close time relation to the liver function test. Fixation of the material is immediate. In some cases the progress of the disease process was studied by comparison of liver sections obtained by serial biopsies with serial function tests.

Laboratory methods. The estimations to be considered are the serum bilirubin, alkaline phosphatase and total serum cholesterol concentrations, together with the total and differential plasma proteins. The tests discussed are the intravenous hippuric acid synthesis test and the intravenous galactose test. Obviously all these investigations and the liver biopsy cannot be performed on the same day; however, it is possible to complete them all in three days. All measurements of time have been reckoned from the second day, on which the biopsy was made.

Serum bilirubin. The total bilirubin of the serum was estimated by the method of Haslewood and King (1937). The upper limit of serum bilirubin in normal subjects has been taken as 1 mg. per 100 ml.

Serum alkaline phosphatase was determined by the method of King and Armstrong (1934). The normal range by this method is 3.7-13. units per 100 ml. Most cases lie between 5 and 10 units, and 10 units per 100 ml. has been taken as the upper limit of normal.

Serum cholesterol was estimated by a modified Liebermann-Burchard reaction (Sackett, 1925). The normal range for this method is 120-130 mg. per 100 ml.

Serum proteins. Total serum protein, serum albumen and serum globulin were estimated by a nesslerization method (King, Haslewood, Delory and Beall, 1937 & 1942). The normal range for this method is: total serum proteins 6-8 g. per 100 ml., serum albumen 3.4-6.0 g. per 100 ml., serum globulin 1.5-3.0 g. per 100 ml., and the albumen-globulin ratio 1.3-4. X

Intravenous galactose tolerance test. The galactose is prepared and sterilized according to the technique of King, Harrison and Delory (1940). 0.5 g. (ml. 50% solution) per kilogram of body weight is injected intravenously to the fasting subject. No toxic effects are encountered. The samples consist of 0.2 ml. of capillary blood which is washed into the requisite amount of isotonic sodium sulphate and sodium tungstate. The specimens are taken before injection and at $\frac{1}{2}$, 1, $1\frac{1}{2}$ and 2 hours after the injection of the galactose. The analysis is performed by the method of King and Aitken (1940).

Interpretation of Results.

In 40 out of 41 subjects without liver disease the galactose had disappeared from the blood within two hours. This two-hour elimination is suitable as a normal standard for general clinical use, but, as it

makes no distinction between the various types of normal curve, for the purposes of comparison, a modification of the galactose time introduced by Barnes and King (1943) was adopted to express the difference between normal and pathological curves.

$$\text{Galactose time} = \frac{a}{(a-b)} \times x$$

where a = 30 minute value for blood galactose

b = last figure for blood galactose before complete elimination from the blood and

x = 30, 60, or 90 minutes, according to whether b is the 60, 90, or 120 minute blood galactose value. The galactose time expresses the rate of disappearance of galactose from the blood in minutes. It gets as near as possible to an average rate of removal of galactose from the blood, and has proved a satisfactory single expression of the test results.

In 41 normal subjects the mean galactose time was 61 (range 30 - 92). The presence of an arbitrary lower limit of 30 minutes to the galactose time prevents more detailed statistical analysis.

Intravenous Hippuric Acid Synthesis Test. The method which popularised hippuric acid synthesis as a measure of liver function was devised by Quick(1932). 6 g. sodium benzoate are given by mouth and hourly urine specimens collected for 4 hours. Normal subjects excrete a total of 4 g. hippuric acid during the test period. The technique has all the disadvantages of oral administration. Gastric stasis, vomiting and faulty absorption will lessen the accuracy of results. Vomiting, in particular, commonly follows the oral administration of sodium benzoate. The collection of

TABLE IV. THE DISTRIBUTION OF THE CASE MATERIAL

Disease	Number of Cases
Acute Hepatitis	84
Epidemic Hepatitis	21
Arsenotherapy Jaundice	54
Serum Jaundice	9
Cirrhosis of the Liver	26
Obstructive Jaundice	49
Haemolytic Jaundice	6
Miscellaneous blood diseases*	3
Hydatid Cyst of liver	4
Amoebic Abscess of liver	1
Weil's Disease (convalescent)	1
Kala Azar	3
Amyloid Disease of liver	4
Secondary malignant disease without jaundice	6
Total	187

* These comprised a case of Gaucher's disease, a case of leucoerythroblastic anaemia and a case of histiocytic medullary reticulosis. All presented hepatomegaly and liver sections showed heavy involvement of the liver in the disease process..

urine over a 4-hour period is often inconvenient. Eventually an intravenous modification was developed (Quick, Ottenstein and Weltchek, 1938). 1.77 g. sodium benzoate in solution are injected intravenously and the urine passed during the first hour after injection collected. This is analysed for hippuric acid by the method of Weichselbaum and Probststein (1938). The normal range for this method has been found to be 0.92-0.84 g. hippuric acid expressed as sodium benzoate (Sherlock, 1945).

Clinical Material.

187 cases of disease involving the liver have been studied. A simple grouping has been made (Table IV - see next page).

Acute Hepatitis. This group includes cases of simple infectious hepatitis ("catarrhal" jaundice), jaundice following the injection of icterogenic mumps convalescent serum or plasma infusions, and jaundice occurring during the course of arsenical therapy for syphilis. Apart from the incubation period and the history of arsenotherapy or the injection of a possibly icterogenic serum, it is extremely difficult to distinguish these three types either from the clinical picture (Turner et al., 1944; Findlay, Martin and Mitchell, 1944) or from the laboratory findings (Hawley et al., 1943; Marshall, 1944; Bradley, Loutit and Maunsell, 1944). The liver pathology is also similar (Mitchell, 1943; Dible and McMichael, 1943; Dible, McMichael and Sherlock, 1943).

The essential pathological lesion is an acute inflammation involving the entire liver. According to the site in the lobule of the maximum change, various types can be distinguished, "zonal", "diffuse," or

"mixed diffuse and zonal". Residual portal fibrotic lesions can also be recognised. These various types blend indefinitely into one another, (Dible et al., 1943). For the purpose of comparing the histological changes with laboratory results, it has been convenient to group the material according to the extent of liver cell damage, i.e. to the probable percentage of surviving hepatic cells (Table V).

TABLE V SHOWING THE HISTOLOGICAL GRADE OF 84 CASES OF ACUTE HEPATITIS

Grade	Probable percentage of surviving liver cells	Number of cases
A	75 - 100	26
B	50 - 75	39
C	25 - 50	14
D	less than 25	5

The grading was done with numbered slides; the patients' names and the results of the various tests were, at the time, unknown. The grading was done by one person. The percentages quoted are only approximate and cannot be treated mathematically. They represent an individual impression. Examples are illustrated (Figs. 4 - 7).

Obstructive Jaundice. In each case there was complete obstruction to the common bile duct which was confirmed by operation or autopsy. Pathological changes in the liver follow very rapidly on occlusion of the common bile duct. The pertinent literature has been very thoroughly reviewed by Cameron and

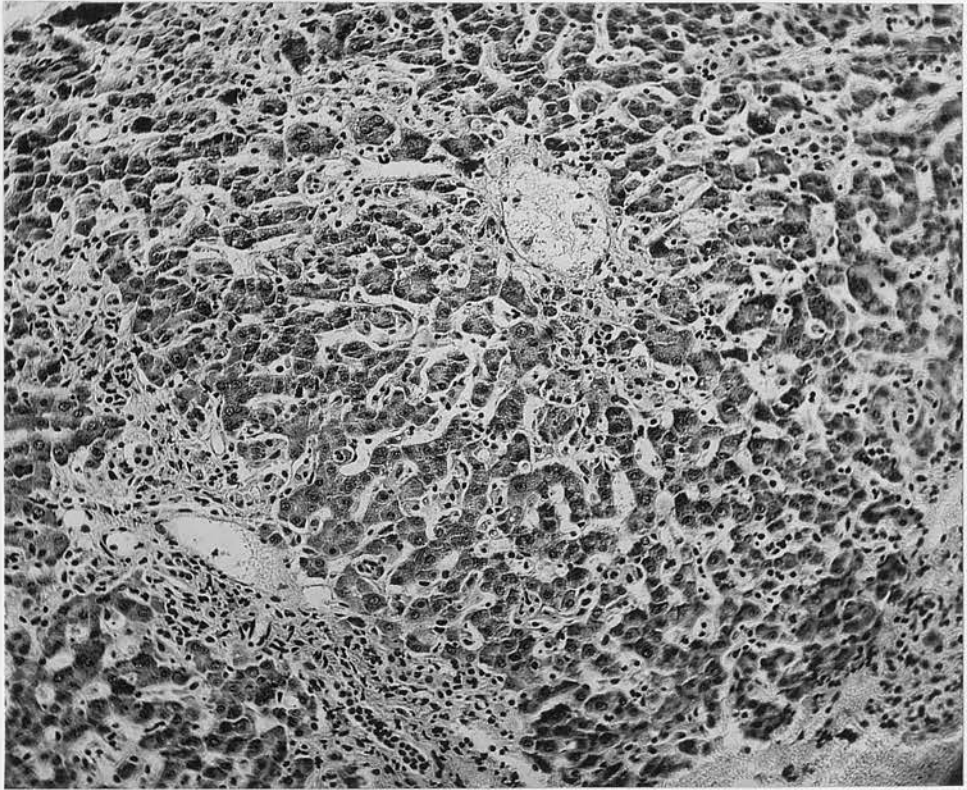


FIG. 4.

Grade A Hepatitis.

S. aged 46. Infectious hepatitis. Jaundiced 7 days.
Serum bilirubin 5.6mg/100ml. Serum phosphatase 23 units/
100ml. Serum cholesterol 250mg/100ml. Total serum protein
6.1g/100ml. Serum albumen 3.2g/100ml. Serum globulin
2.9g/100ml. A/G ratio 1.1. Galactose time 68 minutes.
Hippuric acid excretion 0.85g.
Stained Best's Carmine X 145.

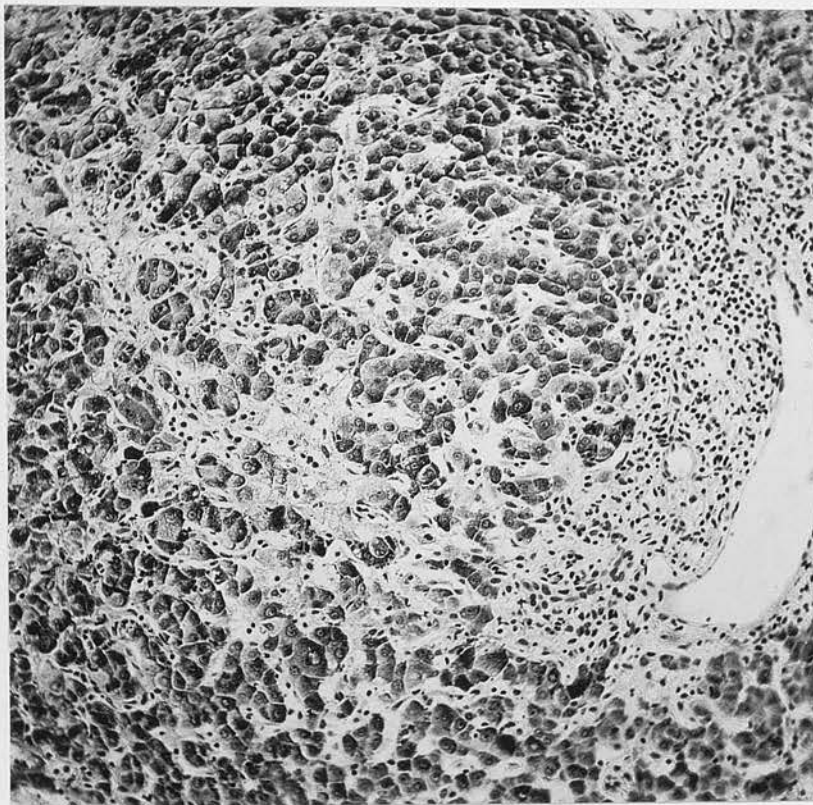


FIG. 5

Grade B Hepatitis.

L. aged 37. 10 days jaundiced. Arsenotherapy jaundice.
Serum bilirubin 6.4mg/100ml. Serum phosphatase 20 units/
100ml. Serum cholesterol 256mg/100ml. Total serum
protein 5.8g/100ml. Serum albumen 3.8g/100ml.
Serum globulin 2g/100ml. A/G ratio 1.9. Galactose
time 60 minutes. Hippuric acid excretion 0.49g.
stained Best's Carmine X 145.

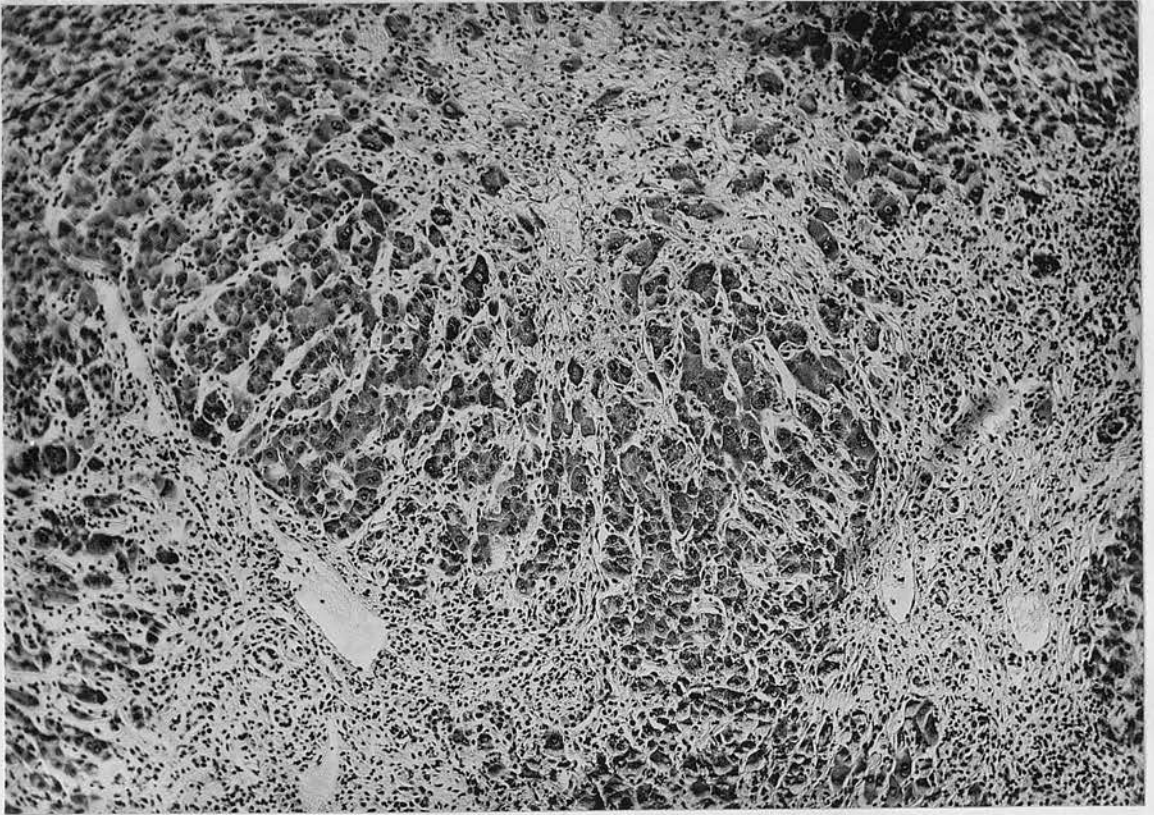


FIG. 6.

Grade C Hepatitis.

P.H. aged 20. Infectious hepatitis. Jaundiced 12 days.

Serum bilirubin 6.9mg/100ml. Serum phosphatase 21 units/100ml. Total serum protein 4.5g/100ml. Serum albumen 2.4g/100ml. Serum globulin 2.1g/100ml. A/G ratio 1.1.

Galactose time 162 minutes. Hippuric acid excretion 0.36g.

Stained Best's Carmine. X 105.

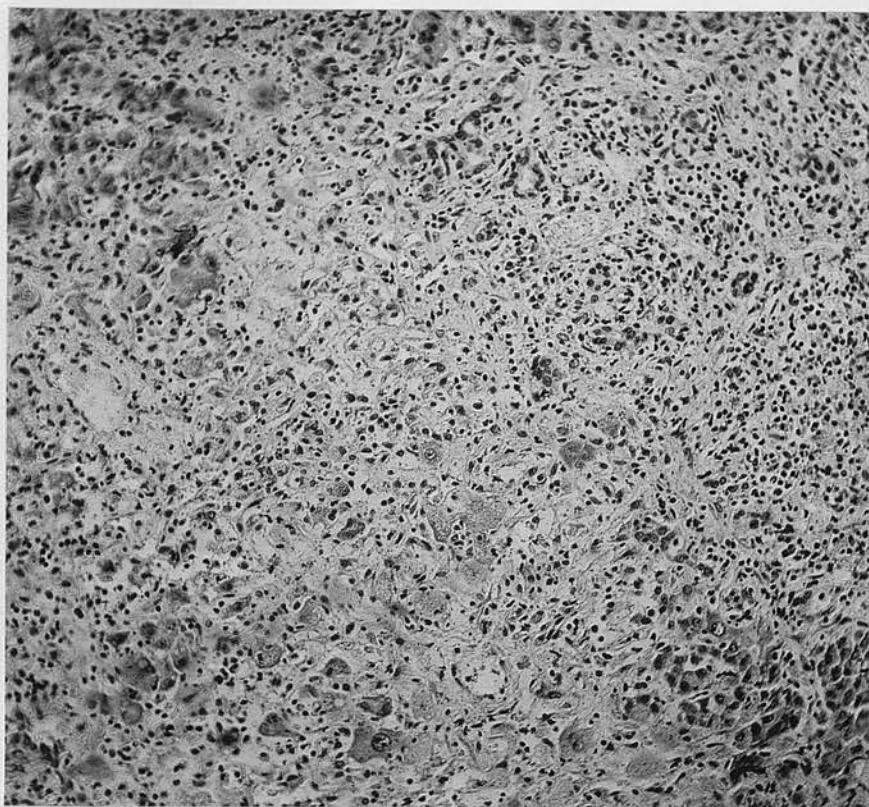


FIG. 7.

Grade D Hepatitis.

M. aged 60. Arsenotherapy jaundice. Jaundiced 9 days.

Serum bilirubin 14.6mg/100ml. Serum phosphatase

17 units/100ml. Serum cholesterol 217mg/100ml.

Total serum protein 6.8g/100ml. Serum albumen 2.8g/

100ml. Serum globulin 4g/100ml. A/G ratio 0.70.

Galactose time 97 minutes. Hippuric acid excretion 0.49g.

Stained Best's Carmine. X 145.

Oakley (1932). These workers found that, in rats, marked histological changes occurred within a few hours of ligation of the common bile duct. Similar results are seen in man (Lieber and Stewart, 1934). In our series also, marked liver changes were noted in each case of obstructive jaundice. Bile pigment is seen in the canaliculi, especially centrally, sometimes precipitated to form the so-called "bile thrombi". The liver cells themselves contain brown granules. Focal bile-stained necroses, usually in relation to the periphery of the lobule, were also seen. Increase of portal tract fibrous tissue, together with proliferation of bile ducts, the epithelium of which was high cuboid, was also noted. Actual disorganization of the liver architecture and massive damage to liver cells was a later feature. The presence of neoplastic metastases was also recorded in those cases coming to autopsy. These provide yet another factor interfering with the normal function of the liver. Some of these changes are illustrated. (Figs. 8 & 9).

Cirrhosis of the Liver. In accordance with modern terminology (de Jong, 1931; Moon, 1932), cirrhosis of the liver has been applied to chronic diffuse liver disease with fibrosis, retrogressive parenchymal changes and regeneration of surviving cells. In addition, we have recognised cases of the latent type in which parenchymal cell damage is minimal and the histological emphasis is on the bands of mature fibrous tissue, sometimes containing proliferating bile ducts, which disrupt the normal architecture of the liver. Nodules of regenerated liver cells may also be observed. This type has been mentioned in the literature,

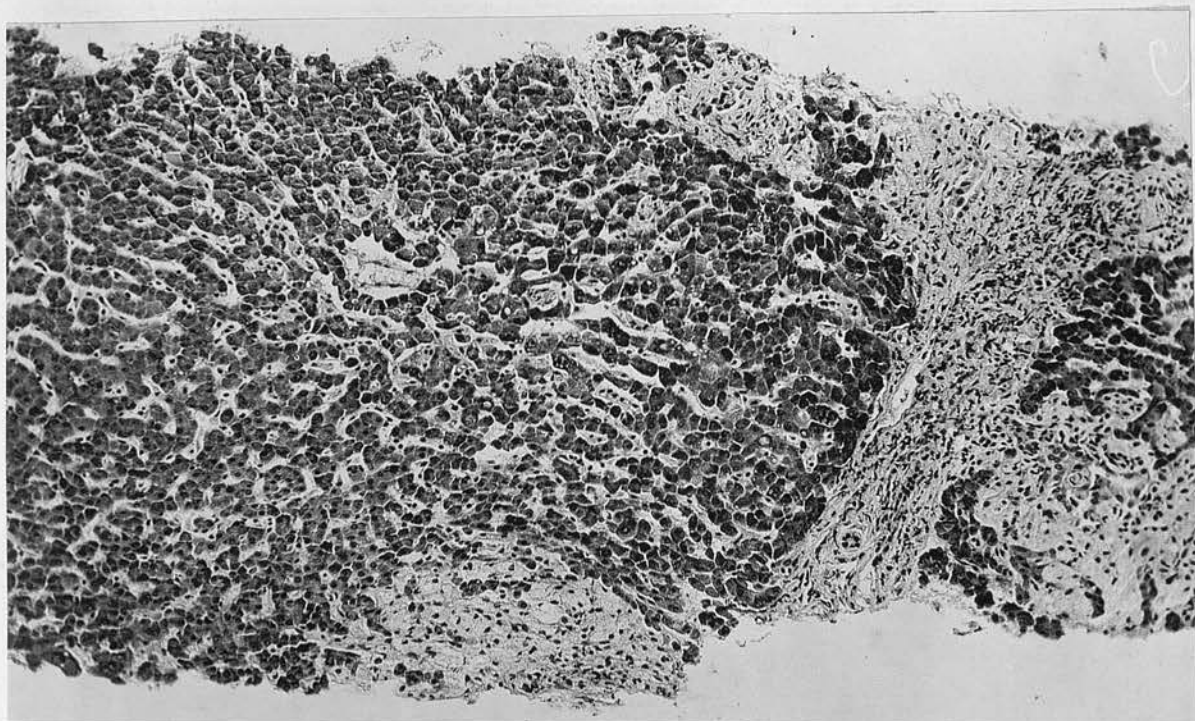


FIG. 8.

Obstructive Jaundice.

Bile stained necroses at periphery of lobule. Increased fibrous tissue and bile duct proliferation in the portal tracts.

O.D. aged 47. Jaundiced 37 days. Serum bilirubin 15mg/100ml. Serum cholesterol 372mg/100ml. Galactose time 47 minutes. Hippuric acid excretion 0.2g.

Stained Best's Carmine X 115.

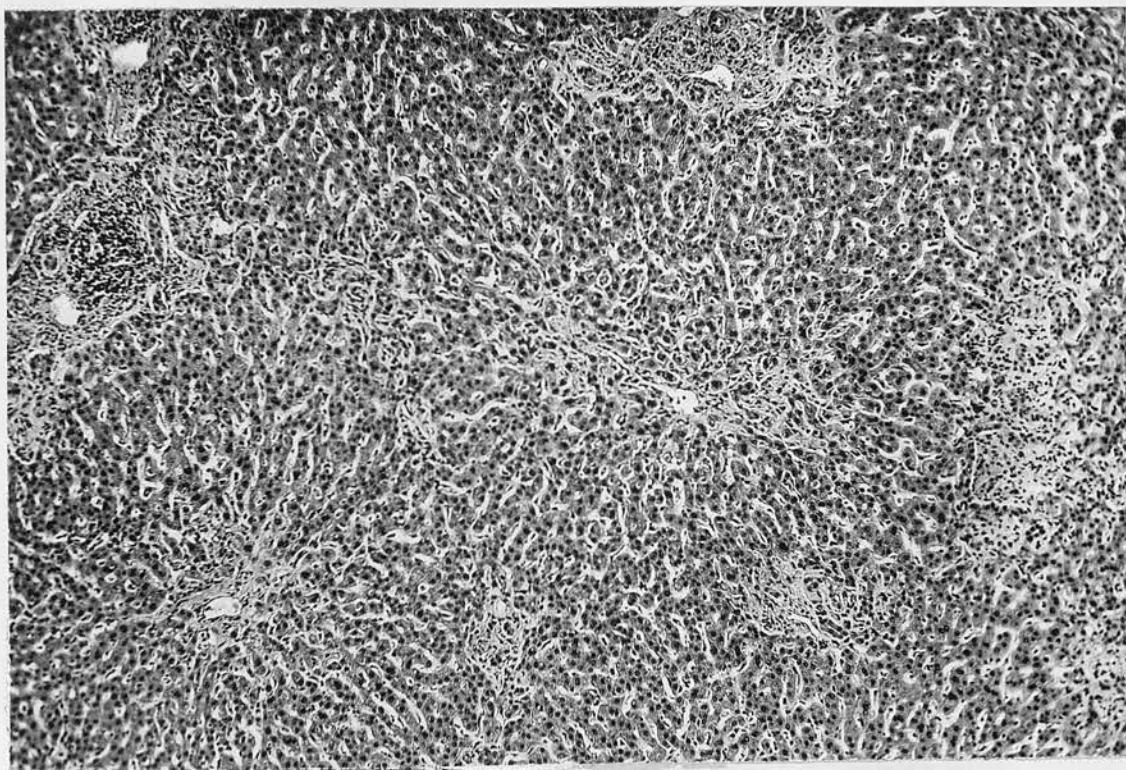


FIG. 9.

Obstructive Jaundice. Early biliary cirrhosis.

W.R. aged 37. Jaundiced 100 days. Serum bilirubin
12.5mg/100ml. Serum phosphatase 33 units/100ml.
Serum cholesterol 256mg/100ml. Total serum protein
6.4g/100ml. Serum albumen 3.1g/100ml. Serum globulin
3.3g/100ml. A/G ratio 0.94. Galactose time 102 minutes.
Hippuric acid excretion 0.4g.
Formalin fixed H.E. X 80.

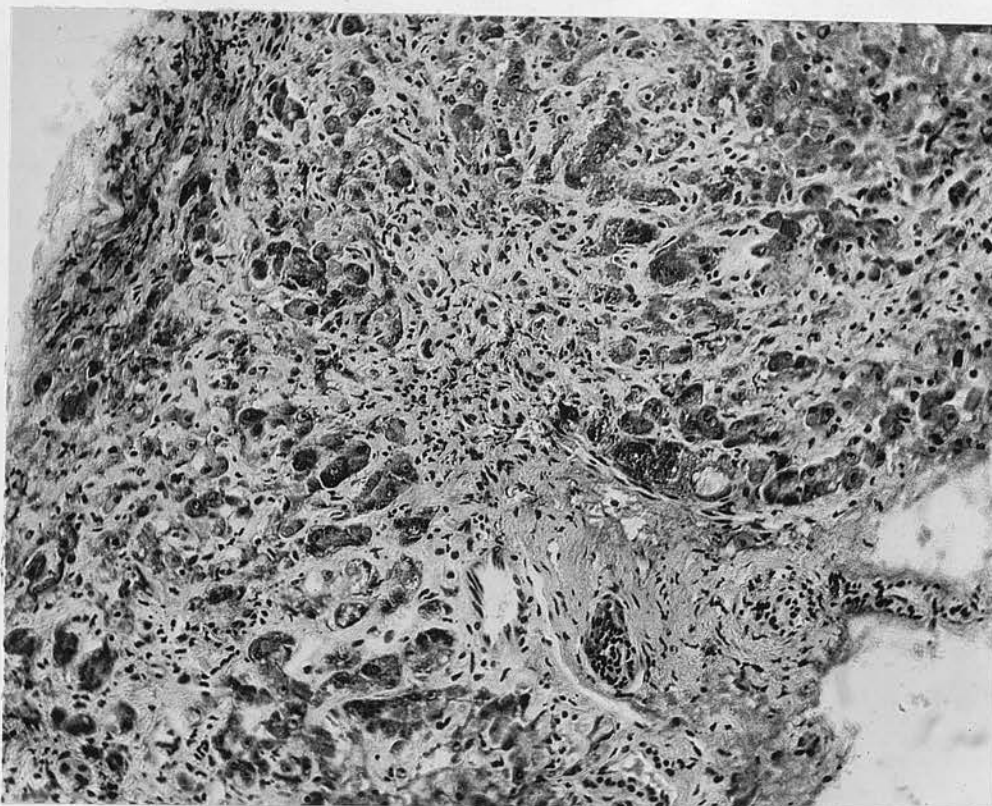


FIG. 10.

Active Cirrhosis.

M.T. aged 40. Serum bilirubin 9.2mg/100ml. Serum phosphatase 19 units/100ml. Serum cholesterol 212mg/100ml. Total serum protein 6.7g/100ml. Serum albumen 2.6mg/100ml. Serum globulin 4.1g/100ml. A/G ratio 0.64. Galactose time 108 minutes. Hippuric acid excretion 0.4g. Stained Best's Carmine X 160.

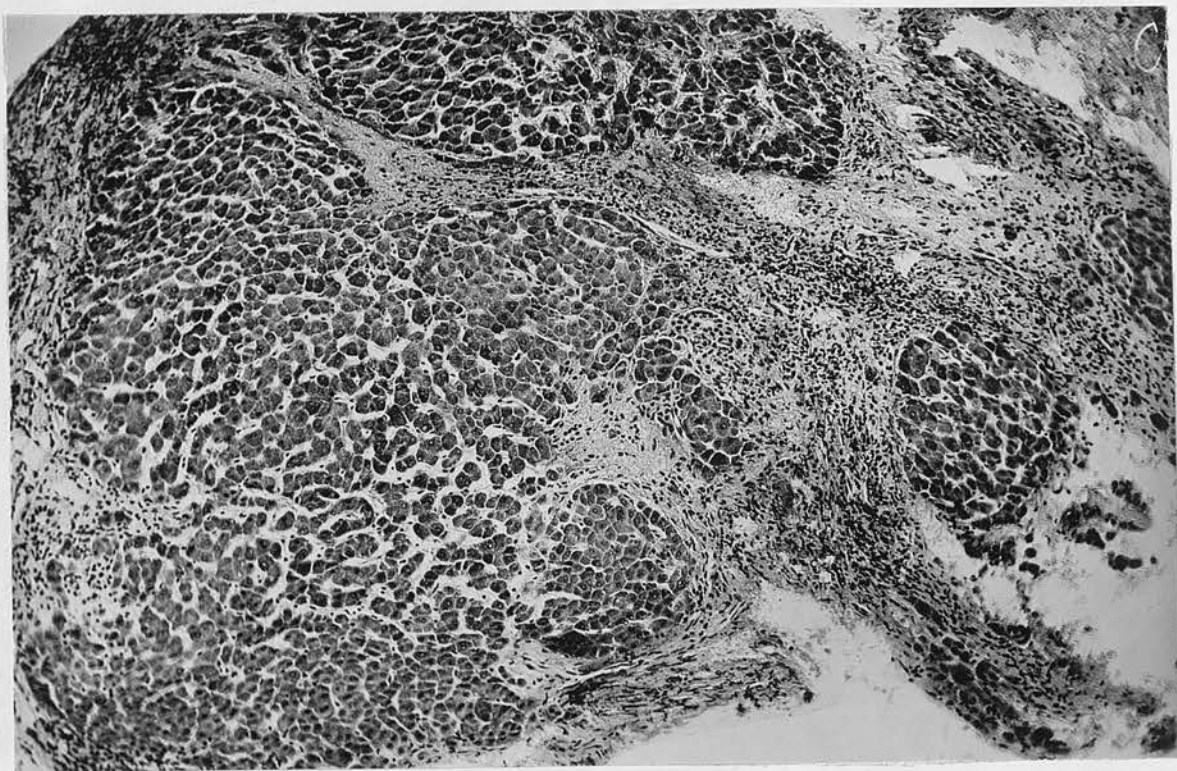


FIG. II.

Latent Inactive Cirrhosis.

A.C. aged 32. Serum bilirubin 0.5mg/100ml. Serum phosphatase 4 units/100ml. Total serum protein 6.8g/100ml. Serum albumen 4.8g/100ml. Serum globulin 2g/100ml. A/G ratio 2.4. Galactose time 33 minutes. Hippuric acid excretion 1.05g.

Stained Best's Carmine X 105

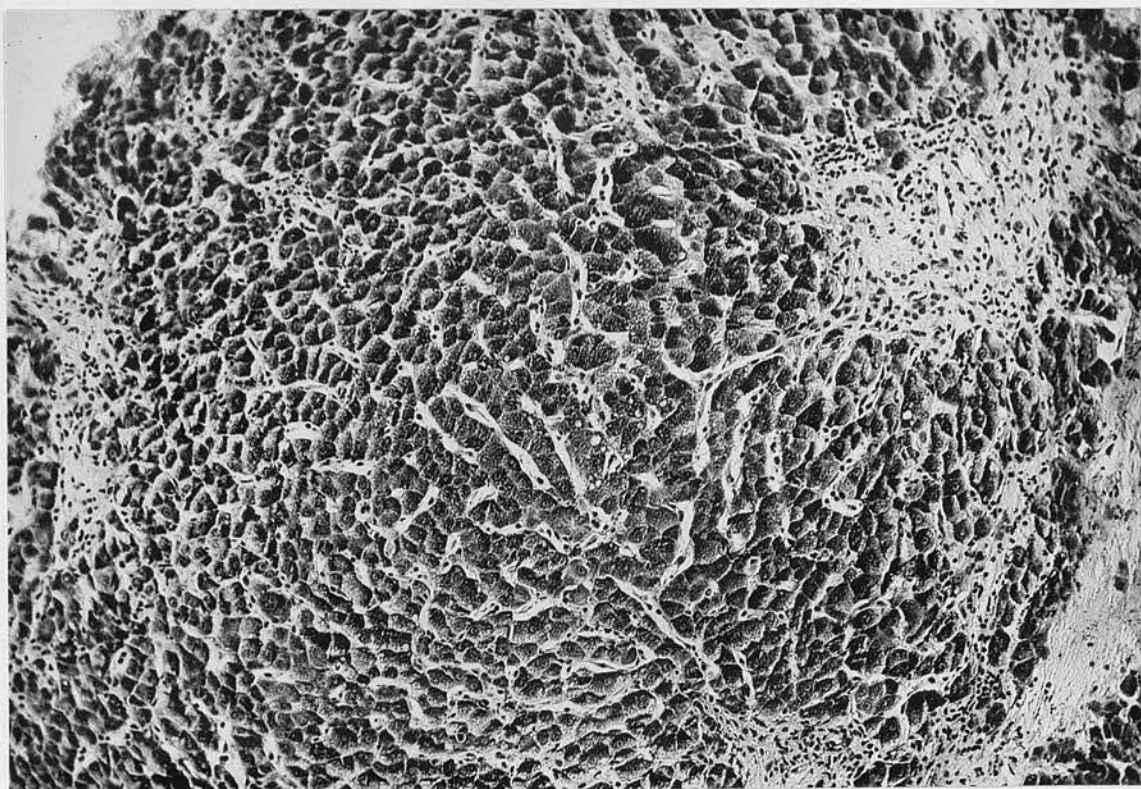


FIG. 12.

Residual portal scarring following hepatitis.

Same case as Fig. 33 days after onset of jaundice.

Serum bilirubin 1.5mg/100ml. Serum phosphatase 12 units/

100ml. Total serum protein 6.2g/100ml. Serum albumen

3.6g/100ml. Serum globulin 2.6g/100ml. A/G ratio 1.4.

Galactose time 46 minutes. Hippuric acid excretion 0.72g.

Stained Best's Carmine. X 135.

(Hall and Ophüls, 1925; McCartney, 1931; Moon, 1932). Some of our cases have followed acute hepatitis and may be analagous to cases of so-called healed subacute liver necrosis (see Mallory, 1933). We have not included in the group recovered cases of hepatitis in which residual fibrous strands are seen in the true anatomical portal tracts without disturbing the essential architecture of the liver. Examples of the three types are illustrated (Figs. 10, 11, 12).

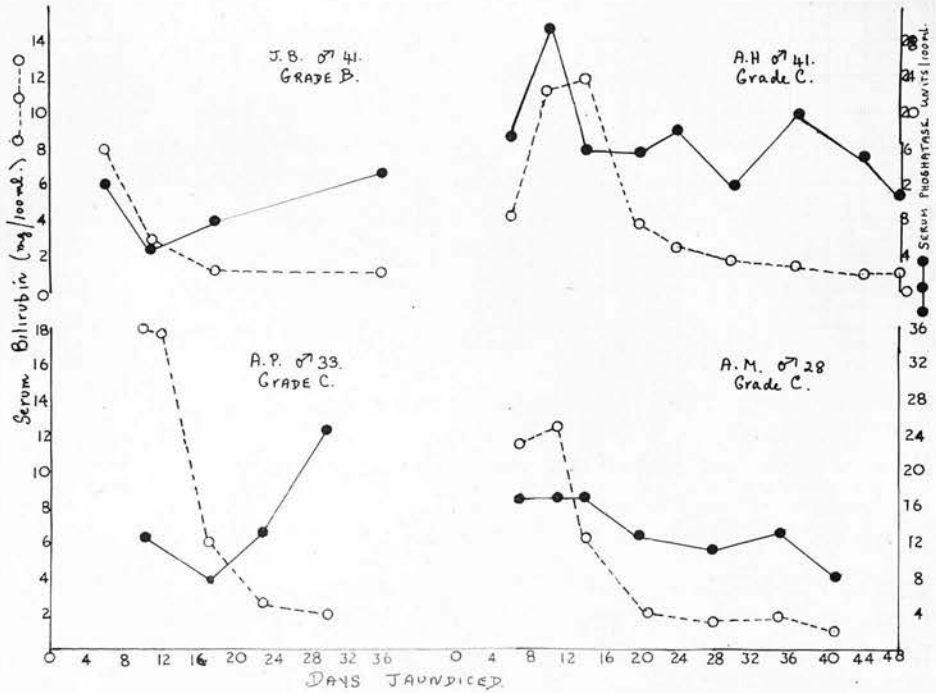
Miscellaneous Diseases. 28 cases. These included a wide variety of conditions in which there is liver involvement. There were examples of haemolytic jaundice, of the reticuloses, of Gaucher's disease and of amyloid disease. Cases of of hydatid cyst, of amoebic abscess and of Kala Azar were also studied. Cases of involvement of the liver in secondary neoplasms without the production of jaundice were included.

CHAPTER IVSERUM BILIRUBINUse of serum bilirubin estimations in the differential diagnosis of liver disease

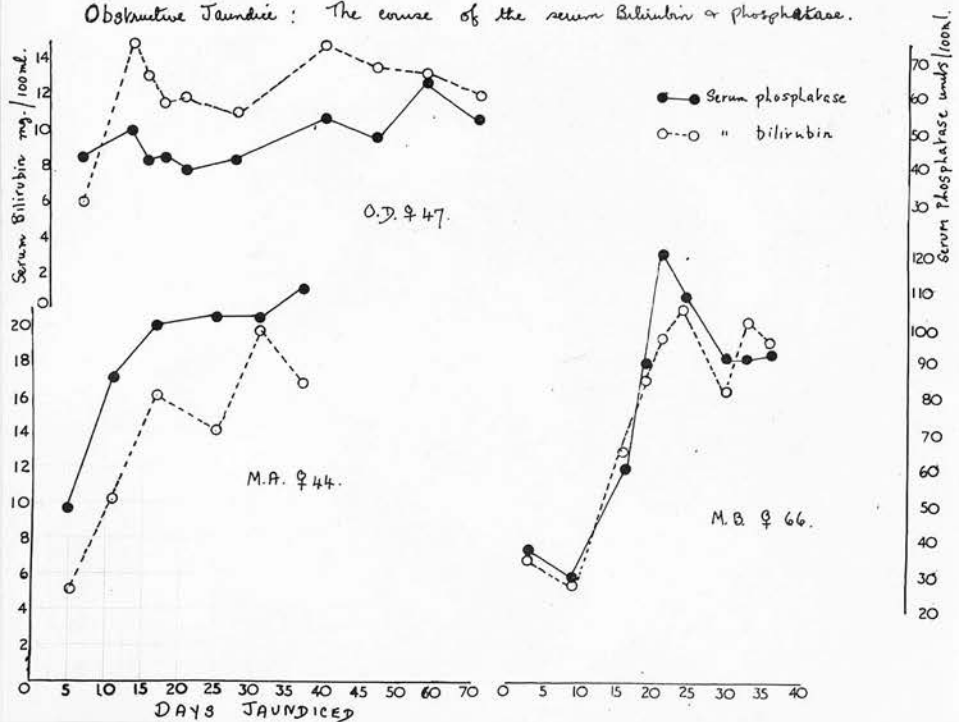
Acute hepatitis and obstructive jaundice. Jaundice is due to an accumulation of bile pigment in the blood. Simply stated, this can be due to excessive haemolysis, to damage to liver cells or to obstruction of the bile duct system. A single estimation of the total serum bilirubin cannot differentiate among these causative factors, and in particular cannot distinguish primary parenchymatous from obstructive jaundice. Before the appearance of frank clinical icterus the diagnosis of infectious hepatitis may be difficult. We have had little opportunity to observe cases during this phase. At that time, however, a slight rise of serum bilirubin may be of considerable value in distinguishing ^{it} from other conditions causing malaise, gastro-intestinal upset and liver (Cameron, 1943; Findlay et al., 1944; U.S.A. Instruction on Jaundice, 1944).

The diagnostic use of the Van de Bergh diazo reaction has fallen into disrepute. Our experience has been that, whatever the cause of the icterus, when the quantitative bilirubin level reaches about 4 mg. per 100 ml., a positive direct Van den Bergh reaction is seen. Claims have been made that parenchymatous may be distinguished from obstructive jaundice by measuring the proportions of direct and indirect bilirubin in the serum (Varela Fuentes, Viana and Recarte, 1934; Bengolea, Suárez and Raíces, 1935; Franké, 1936). Other workers have been unable to confirm these findings. (Heilbrun

Acute Hepatitis: the course of the serum Bilirubin & phosphatase.



Obstructive Jaundice: The course of the serum Bilirubin & phosphatase.



and Hubbard, 1940; Sepulveda and Osterberg, 1943b.). We employed the technique of Sepulveda and Osterberg (1943a) but difficulties in the chloroform extraction of the indirect reacting bilirubin and some uncertainty concerning the rationale of the extraction led to its abandonment. The technique has recently been criticised (Ducci and Watson, 1945). If serial serum bilirubin readings are available during the course of the disease, then the estimation may be of some diagnostic value. The patient is rarely admitted to hospital during the pre-icteric phase of acute hepatitis; the disease, moreover, is usually of limited duration and the trend of the bilirubin readings is downward. The levels recorded during the first week in hospital are usually the highest. Fig. 13 shows the usual trend of bilirubinaemia in the jaundiced phase of acute hepatitis. In all 4 cases illustrated a second liver biopsy before discharge showed complete histological recovery. In only 3 out of 30 cases of hepatitis in which the bilirubinaemia was followed to below 2 mg. per 100 ml. was an increase of jaundice recorded after the first 7 days in hospital.

In the 6 cases of unrelieved obstruction which proceeded to necropsy the bilirubin steadily rose for about the first three weeks of jaundice; the level then fluctuated, the trend always being upward (Fig. 14). If the bilirubinaemia increases for three weeks, the course is unlikely to be hepatitis.

Hepatic cirrhosis. Cirrhosis has often been reported in the absence of jaundice (Rolleston and McNee, 1929; Weiss, 1935; Epstein and Greenspan 1936; Bassett

TABLE 6.

*
THE SERUM BILIRUBIN IN 187 CASES OF
LIVER DISEASE.

Diagnosis	Mean	S.E. Mean	Range	Range of values in mg./100 ml.						Totals
				0-0.9	1-3.9	4-6.9	7-9.9	10-13	Greater than 13	
Acute hepatitis	6.77	0.45	18.0-1.1	0	25	24	16	9	10	84
Obstructive jaundice	9.1	0.99	34-2.0	0	9	16	8	4	12	49
Cirrhosis										
Active	5.0	0.85	11.2-1.2	0	8	4	4	1	0	17
Latent	0.73	0.08	1.2-0.5	7	2	0	0	0	0	9
Haemolytic jaundice	2.37	0.24	3.0-1.0	0	6	0	0	0	0	6
Misc. blood diseases	0.5			3	0	0	0	0	0	3
Hydatid Cyst	0.5			4	0	0	0	0	0	4
Amoebic Abscess	5.1			0	0	1	0	0	0	1
Weill's disease	2.3			0	1	0	0	0	0	1
Kala Azar	0.5			3	0	0	0	0	0	3
Amyloid disease	0.5			4	0	0	0	0	0	4
Secondary malignant disease	0.71	0.15	1.5-0.5	5	1	0	0	0	0	6

*

Normal serum bilirubin up to 1 mg./100 ml.

Althausen and Coltrin, 1941). We have observed 26 instances of histologically confirmed cirrhosis: of these 9 were noted to have a bilirubin of less than 1.5 mg. per 100 ml. The absence of bilirubinaemia does not exclude a diagnosis of hepatic cirrhosis. In the presence of jaundice the measurement of serum bilirubin, whether on one occasion only or during the course of the disease, gives little help in distinguishing a cirrhotic from an obstructive jaundice. The maximum height is very variable and the level tends to fluctuate, in some instances resembling the late stages of an obstructive jaundice; it is unusual, however, for the bilirubin to rise above 12 mg. per 100 ml. in uncomplicated cirrhosis.

Miscellaneous diseases affecting the liver. Scrutiny of Table 6 shows that the liver can be involved in diverse disease processes without raising the plasma bilirubin.

Use of the serum bilirubin as a guide to the severity of liver damage.

Acute hepatitis. There are divergent opinions regarding the use of the serum bilirubin level as an index of the severity of a case of acute hepatitis. Turner, Snively, Grossman, Buchanan and Foster (1944) have analysed over four thousand cases of "yellow fever serum" jaundice, and state that the cases of the greatest clinical severity and of the longest duration had icteric indices of more than 120 units. Higgins, O'Brien, Stewart and Witts (1944) believe that the serum bilirubin is a reliable guide to the immediate prognosis in hepatitis.

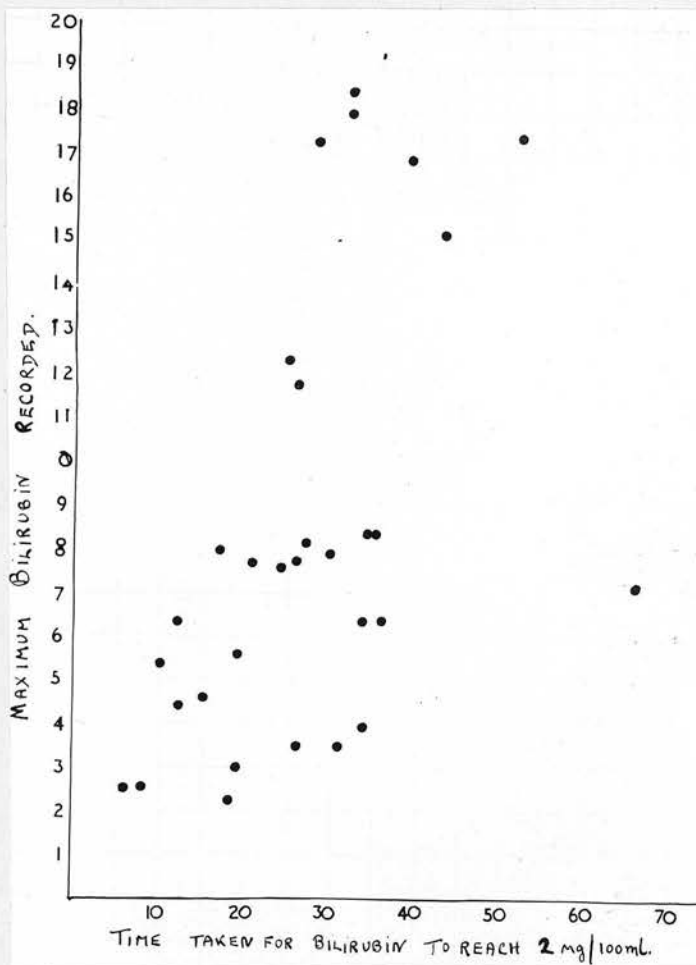
TABLE VII. THE SERUM BILIRUBIN CORRELATED WITH THE HISTOLOGICAL SEVERITY OF
ACUTE HEPATITIS

Histological Grade	Mean	S.E. Mean	Range	Range of values in mg./100 ml.					Totals	
				0-1	1-3.9	4-6.9	7-9.9	10-13		Greater than 13
A	3.4	0.62	12.8-1.1	0	15	9	1	1	0	26
B	7.0	0.4	17.4-2.2	0	10	11	11	3	4	39
C	9.7	0.88	18.0-5.3	0	0	4	4	4	2	14
D	14.4	0.98	17.0-9.8	0	0	0	0	1	4	5

Lucké (1944), in a study of fatal cases, mentions that as the end phase occurs, the icteric index fluctuates, but tends to rise particularly in the final period. Findlay and his co-workers (1944), however, state that the icteric indices showed wide variation and gave no indication of the severity of the disease. Gray (1945) states that the intensity of jaundice appears to be no indication of the severity of the underlying liver lesion. Our observations show that, as the damage to the liver cells increases, so the mean serum bilirubin of the group rises (Table 7).. The wide range and large standard deviation in each group illustrate the individual variation from case to case. The statement, therefore, can only be a generality. It is, however, true that a plasma bilirubin of more than 10 mg. per 100 ml. is usually associated with one of the severer grades of liver damage. Of 26 cases of histological grade "A" only one had a higher level. The reverse, namely that a low bilirubin is associated with mild liver damage, is not true. Any attempt at explanation of these apparently contradictory findings involves a consideration of the causation of the bilirubinaemia in acute hepatitis, a matter beyond the scope of this paper. In 30 cases, initially observed during the first two weeks of jaundice, the plasma bilirubin was followed at intervals of not more than seven days, until a level below 2 mg. per 100 ml. was reached. The bilirubin of all the cases of minimum histological severity (Grade A) fell to below 2 mg. in less than 3 weeks. The more severe cases required longer. There was a correlation between the time required and the initial histological severity of hepatitis. Similarly, the

cases with the highest initial serum bilirubin took the longest time to reach 2 mg. per 100 ml. (Fig.15).

15.



The correlation coefficient between the maximum bilirubin and the duration of bilirubinaemia is 0.78 for 30 cases. This is statistically significant. The bilirubin level does not show a constant rate of fall as recovery proceeds. The initial fall is very rapid, especially from the higher figures. The eventual fall to normal is slower (Fig.13). This can be correlated with the histological course of the disease. The liver cells possess a remarkable power of regeneration, and recovery, once initiated, proceeds rapidly. In

the majority of cases the reticular framework of the liver has throughout the acute phase remained virtually intact. With recovery, the multiplying liver cells fall into place in regular columns. The centres of the lobules are the last to reform. There is coincident restitution of the bile canaliculi and the bilirubin can therefore be rapidly excreted in the bile. The fact that the serum bilirubin may remain slightly elevated (1-2 mg. per 100 ml.) for a week or two after apparent clinical recovery does not imply the development of a subacute or chronic hepatic lesion. The liver histology is nearly always normal in these instances and the excess bilirubinaemia usually soon disappears, (see Figs. 7 & 12). This later feature has also been commented on by Hayman and Read (1945) in yellow fever serum jaundice.

Summing up, therefore, in acute hepatitis a high serum bilirubin is usually associated with a severe degree of liver damage. The cases with the greatest initial bilirubinaemia take the longest time for the disappearance of the excess bilirubin from the plasma. A marked initial degree of liver cell damage does not necessarily indicate a slow histological recovery. The power of the liver cells to regenerate is enormous, and once started it is usually rapidly completed.

Obstructive jaundice. The bilirubinaemia of obstructive jaundice depends on the blockage of the common bile duct. The secondary changes produced in the liver itself play little part in its production. The serum bilirubin level, therefore, gives little help in determining the extent of liver damage. Cases in which liver damage was pronounced have been selected.

The liver in these instances showed gross biliary cirrhosis and/or were riddled with neoplastic metastases. This serum bilirubin has been compared with that found in cases in which the obstruction to the common bile duct was associated with minimal liver changes. Both groups were of approximately the same duration. The mean bilirubin in each group showed no significant difference.

Cirrhosis of the liver. In 8 of the cases of hepatic cirrhosis in which the serum bilirubin was less than 1 mg. per 100 ml. there were no definite symptoms referable to the liver. 17 cases with marked bilirubinaemia did show such symptoms. 12 of them died while in hospital. The actual height of the serum bilirubin in these cases did not serve as a means of predicting the fatal outcome; neither did it correlate with the apparent extent of liver damage as seen by biopsy or at autopsy. This lack of correlation contrasts with the findings in acute hepatitis. In this latter condition, in the acute phase, degeneration is the main cellular change observed. In cirrhosis, on the other hand, necrosis and regeneration are proceeding side by side. The height of the bilirubinaemia is mainly dependent upon the balance between these processes; it is not, in this condition, dependent upon cell damage alone. Bilirubinaemia in cirrhosis, if present, is an indication of inadequately compensated destruction of liver cells or or disruption of the normal lobular architecture and bile duct system producing an intra-lobular obstruction to the excretion of bile.

Miscellaneous diseases. In the preceding paragraph the changes in the liver essential for jaundice other than haemolytic have been mentioned. These changes are diffuse liver cell damage and profound disorganisation of the intrahepatic bile duct system. The tremendous reserve of power of the liver compensates for almost any localized lesion, especially if chronic. This explains how such conditions as hydatid cysts or secondary carcinomatous deposits which do not obstruct a main bile duct are associated with a normal plasma bilirubin concentration. This applies also to such conditions as Kala Azar and the reticuloses in which the liver cells and biliary passages are essentially normal.

Chapter V.

Serum Alkaline Phosphatase.

Use of serum phosphatase estimations in the differential diagnosis of liver disease.

Acute hepatitis and obstructive jaundice. In 1930 Roberts observed that a raised serum phosphatase occurred in obstructive jaundice. This increase has since been corroborated both in experimental animals (Bodansky and Jaffe, 1933; Armstrong and King, 1934; Freeman, Chen and Ivy, 1938; Schiffman and Winkelman, 1939; Gutman, Olson, Gutman and Flood, 1940; Sehra, Chopra and Mukerji, 1941;) and in man (Roberts, 1930 & 1933; Greene, Shattuck and Kaplowitz, 1934; Herbert, 1935; Rothman, Meranze and Meranze, 1936; Cantarrow and Nelson, 1937). However, it was soon realised that liver cell damage also caused a rise in the serum phosphatase, usually to a lesser degree than that following occlusion of the common bile-duct. The rise was noted in experimental liver injury (Bodansky and Jaffe, 1933; Armstrong and King, 1935; Rothman et al., 1936; Sehra et al., 1941; Drill and Ivy, 1944) and also in human hepatitis (Bodansky and Jaffe, 1933; Herbert, 1935). This rise with liver cell damage, even though less than that occurring in obstructive jaundice, means that some overlap of results will occur. This had led to the condemnation of the

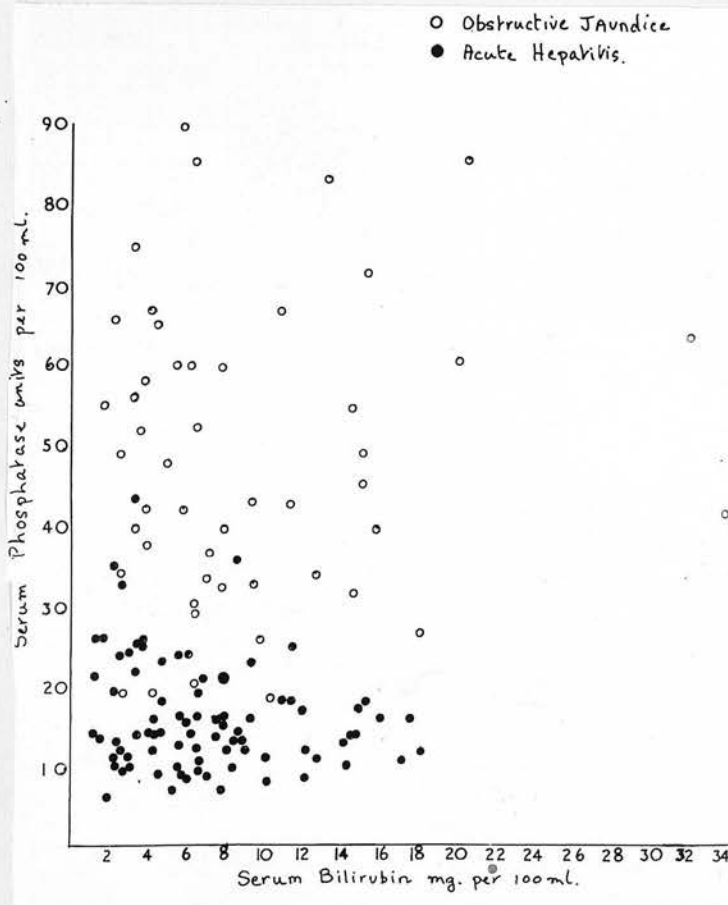
TABLE VIII. THE SERUM ALKALINE PHOSPHATASE* IN 186 CASES OF LIVER DISEASE

Diagnosis	Mean	S.E. Mean	Range	Range of values in units/100 ml.										Totals
				0-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-50	Greater than 50	
Acute Hepatitis	15.6	0.77	43-7	11	35	19	11	4	2	1	1	0	0	84
Obstructive Jaundice	48.6	2.6	90-18	0	0	3	1	3	7	2	6	4	23	49
Cirrhosis														
Active	16.4	1.9	36-7	1	7	4	2	0	1	1	0	0	0	16
Latent	12.0	2.7	25-4	4	2	1	1	1	0	0	0	0	0	9
Haemolytic														
Jaundice	5.7	0.14	7-4	6	0	0	0	0	0	0	0	0	0	6
Misc. Blood														
Diseases	7.3			3	0	0	0	0	0	0	0	0	0	3
Hydatid														
Cyst	10.6		16-3.3	1	2	1	0	0	0	0	0	0	0	4
Amoebic														
Abscess	31			0	0	0	0	0	1	0	0	0	0	1
Weil's														
Disease	16			0	0	1	0	0	0	0	0	0	0	1
Kala														
Azar	12		18-9	1	1	1	0	0	0	0	0	0	0	3
Amyloid														
Disease	14		21-10	0	2	1	1	0	0	0	0	0	0	4
Secondary														
Malignant														
Disease	11.6		23-5	3	1	0	1	0	0	0	0	0	0	5

* Normal serum alkaline phosphatase 3.7 - 13.1 units/100 ml.

method as a diagnostic procedure (Greene et al., 1934; Cantarrow and Nelson, 1937; Giordano, Wilhelm and Prestrud, 1939; Gutman et al., 1940).

16.



Our observations are seen in Table 8 and Fig. 16. If an arbitrary level of 30 units is decided upon (3 times the normal upper limit) then only 4 of

84 cases of acute hepatitis fall above this level and only 7 of 49 cases of obstructive jaundice fall below it. In our series this proved a better dividing line than the 25 King-Armstrong units recommended by MacLagan (1944a) or the 10 Bodansky units of Gutman and Hanger (1941)

As Rothman et al. have suggested, if more than one phosphatase estimation is available, then accurate diagnosis is further helped. In the course of obstructive jaundice the level usually rises progressively. The figures on which the above data were based were the first serum phosphatase of each patient recorded on admission to hospital. In 17 of the cases further readings were available; if the mean of the first observations is compared with the mean of the highest recordings, then the mean rises from 52 to 80. The course of the serum phosphatase in 3 cases of unrelieved obstruction is shown graphically. Fig. 14. It is therefore apparent that if at first serum phosphatase is equivocal, then a second, a week later, may be so high that acute hepatitis is placed out of reckoning.

In acute hepatitis the serum phosphatase usually falls with recovery. Frequently however, there are fluctuations which cannot be correlated

with the serum bilirubin, with the liver function tests studied or with the changes in liver pathology. Fig 13. The levels, however, never rise to those found in obstruction. Neither in obstructive jaundice nor in acute hepatitis could any correlation be established between the height of the serum bilirubin and that of the phosphatase. Fig. 16.

Cirrhosis of the liver. Recent work has tended to minimise the value of serum phosphatase estimations in the differentiation of cirrhosis from other causes of jaundice. MacLagan (1944a) states that values greater than 35 units are frequent in cirrhosis. Higgins et al. (1944) also report high values in this condition. Our findings, however, agree with those of Gutman et al. 1940. Cirrhosis, whether active or latent, is rarely found with a serum phosphatase greater than 30 units per 100 ml. (Table 8). The means are similar to those of the acute hepatitis group and, as in that condition, the plasma phosphatase is of considerable value in the distinction of these cases from obstructive jaundice.

Miscellaneous diseases affecting the liver.

It has been suggested that in the absence of jaundice a raised serum phosphatase may result from disturbed liver metabolism.

Carcinoma of the liver, liver abscess and amyloid

disease are mentioned as such conditions.

(Gutman et al., 1940; Higgins et al., 1944; MacLagan, 1944). We have been unable to confirm these findings. Localised liver lesions, for example carcinomatous metastases and hydatid cysts or diseases of the reticuloendothelial system in the liver, such as kala azar, are not usually associated with a raised plasma phosphatase. In the absence of jaundice the estimation of the serum phosphatase seems of no value in the diagnosis of liver diseases.

Use of serum phosphatase estimation in the determination of the extent of liver cell damage.

Acute Hepatitis. In experimental animals estimation of the serum phosphatase has been recorded as of value in detecting liver damage due to chemical poisons (Drill and Ivy, 1944). Armstrong and King (1935) suggest that the increase may be proportional to the degree of liver damage. The estimation might therefore be useful in assessing the extent of the acute diffuse liver damage present in acute hepatitis. However, Higgins and his co-workers (1944) record no close correlation between the serum phosphatase and the clinical condition of the patient, and our biopsy studies have not shown a relation between the degree of the histological severity and the serum phosphatase level. In all the

histological grades there was a moderate increase in the plasma phosphatase (Table 9 on next page). There was no significant difference between the means for the various groups. These findings suggest that the liver cell is not the main source of the increase in phosphatase encountered in parenchymatous jaundice.

Hanger and Gutman (1940) found that a higher serum phosphatase was associated with arsenotherapy jaundice than with simple epidemic jaundice. This was attributed to the presence of cholangitis and pericholangitis in the arsenotherapy cases. We have not observed this histological picture. The phosphatase in the arsenotherapy group was not higher than that of simple infectious hepatitis. The mean for 54 arsenotherapy cases is 15.8 (range 35-6) and 21 simple infectious hepatitis cases 15.0 (range 43-7).

In 30 cases the serum phosphatase was followed during the course of the disease. The enzyme did not show the constant fall characteristic of the bilirubin level. In 19 of 30 cases of acute hepatitis the serum phosphatase level fluctuated during the recovery period. A longer time was required to reach normality than that needed for the serum bilirubin. When the serum bilirubin had reached 2mg. per 100ml., in only 10 out of 30 cases was a serum phosphatase of

TABLE 9.

THE SERUM PHOSPHATASE CORRELATED WITH THE HISTOLOGICAL
SEVERITY OF ACUTE HEPATITIS

Histological grade	Mean	S.E. Mean	Range	Range of values in units/100 ml.								Totals
				0-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	
A	15.08	1.07	25-7	3	11	6	5	1	0	0	0	26
B	17.43	0.63	43-7	6	16	8	4	2	1	1	1	39
C	17.0	1.78	33.8	2	4	4	2	1	1	0	0	14
D	13.0	0.84	17-10	0	2	3	0	0	0	0	0	5

10 units recorded. On discharge from hospital 12 cases still showed a raised plasma phosphatase. In the 7 cases in which opportunity occurred to follow the level over the succeeding twelve months, all eventually showed normal values. In general, the cases in which initial histological damage to the liver was greatest took the longest time for the plasma phosphatase to reach normal. There were, however, exceptions to this rule. The slowly falling phosphatase was not reflected in the progress of the histological recovery. In 14 cases in which the phosphatase varied during recovery a follow-up biopsy ~~was~~ performed during the recovery phase and the usual progress of rapid cellular restoration was confirmed. The serum phosphatase, therefore, is of no value in assessing the severity of a case of acute hepatitis.

Obstructive jaundice. The level of the serum phosphatase in obstructive jaundice depends on the blockage of the common bile duct and does not reflect the secondary changes occurring in the liver. Progressive liver damage in obstructive jaundice, as revealed by serial hepatic biopsies, did not influence the upward trend of the serum phosphatase during the course of the illness. The level ~~is~~ not significantly different where massive hepatic metastases are associated with the obstruction. Extreme cachexia does not influence

the very high levels reached in the last stages of obstructive jaundice due to carcinoma.

Cirrhosis of the liver. Where liver cell necrosis is noted, the serum phosphatase is moderately raised. In this small series no correlation existed between the degrees of increase and the extent of the cell damage. In 4 out of 9 cases of latent cirrhosis the phosphatase was above 10 units per 100ml. In 2 of these this was the only biochemical abnormality demonstrated. Until more is known of the mechanism of serum phosphatase changes in liver disease, any explanation of the increase encountered in some cases of cirrhosis must be hypothetical. A possible factor is that the presence of fibrous bands, in the absence of parenchymal damage, may so disorganise the intrahepatic biliary system that an obstruction to the biliary radicles is produced and hence a rise in phosphatase.

Miscellaneous disease involving liver. In the various conditions studied (Table 8) the serum phosphatase was of little value in estimating the extent of liver involvement in the disease process.

SERUM CHOLESTEROL

The clinical usefulness of the estimation of blood cholesterol is minimised by the wide range of the substance in the blood of normal subjects.

The pertinent literature has been recently reviewed by Jones (1942). This author points out that apart from the wide normal range the level depends upon whether oxalated plasma, serum or whole blood is used for the analysis. This leads to difficulties in comparing the results obtained by different workers. The level also depends on certain pathological differences apparently unrelated to the liver. Fever, malnutrition and anaemia are known to depress the blood cholesterol. These conditions are frequent concomitants of liver and biliary tract disease.

Our results were practically valueless in the investigation of liver diseases, and the estimation of total serum cholesterol has been eliminated from the later cases in this series. The measurement of the proportion of esterified to free cholesterol, a more constant figure in normal subjects, might have provided more useful information, but this was not added to the battery of tests already in use.

Use of the serum cholesterol estimations in the differential diagnosis of liver disease.

Acute hepatitis and obstructive jaundice. Ever since the original observation of Austin Flint Jr. in 1862 that the level of the blood cholesterol might be used to differentiate the types of jaundice, interest has developed in the study of the relationship of blood cholesterol to liver function. It is

49

usually reported that obstruction to the common bile duct is associated with a raised level; parenchymatous liver disease, on the other hand, usually results in a diminished concentration. Our results are seen in Table VII. The difference between the means for the acute hepatitis and obstructive groups is statistically significant. This, however, gives a false impression of the diagnostic potentialities of the method. The actual overlap between the results encountered in the two groups is seen in Table X on following page. 18 out of 39 cases of obstructive jaundice had levels of less than 260 mg. per 100 ml.; 14 out of 53 cases of acute hepatitis were above the normal upper limit of 230 mg. per 100 ml. Further serum cholesterol reading during the course of the illness added little diagnostic information. In a case of jaundice, if the serum cholesterol is greater than 300 mg. per 100 ml., the cause of the icterus is probably biliary obstruction. Apart from this, estimation of the total serum cholesterol has little place in the diagnosis of jaundice. A statistically significant correlation could not be established between serum cholesterol and serum phosphatase or serum bilirubin in either acute hepatitis or obstructive jaundice.

Cirrhosis of the liver. Cates (1941) reported that 9 out of 16 cases of cirrhosis had a diminished total blood cholesterol with a change in the proportion of free to esterified fractions. Epstein and Greenspan (1936) found a normal blood cholesterol in cirrhosis except when jaundice supervenes as a result of intercurrent acute hepatic degeneration or terminally cholaemia; the level then behaves as in primary liver degenerations. We observe the total serum

TABLE X

*
THE SERUM TOTAL CHOLESTEROL IN 128 CASES OF
LIVER DISEASE.

Diagnosis	Mean	S.E. Mean	Range	Range of values in mg/100 ml.								Greater than 350	Totals
				110	140	170	200	230	260	290	320		
				to	to	to	to	to	to	to	to		
				139	169	199	229	259	289	319	349		
Acute hepatitis	193	6	285-110	5	12	12	9	12	2	0	0	0	53
Obstructive jaundice	262	10	550-167	0	1	2	5	9	3	4	4	4	29
Cirrhosis													
Active	202	13	327-120	2	2	5	2	2	0	1	1	0	15
Latent	182	12	268-140	0	2	3	2	0	1	0	0	0	8
Haemolytic jaundice	182		225-131	1	0	2	1	0	0	0	0	0	4
Misc. blood diseases	184			0	0	3	0	0	0	0	0	0	3
Hydatid cyst	255		302-118	1	1	1	0	0	0	1	0	0	4
Weill's disease	260			0	0	0	0	0	1	0	0	0	1
Kala Azar	183		189-177	0	0	3	0	0	0	0	0	0	3
Amyloid disease	214		250-173	0	0	2	0	2	0	0	0	0	4
Secondary malignant disease	200		246-147	0	2	0	0	2	0	0	0	0	4

*

Normal serum total cholesterol 120 - 230 mg./100 ml.

cholesterol in 23 cases of hepatic cirrhosis; all except five fell within the normal range. The exceptions were above normal; two occurred in alcoholic subjects. The serum phosphatase was not raised. Acute and chronic alcoholism are known to raise the plasma cholesterol (Lichtmann, 1942). The levels were not lower in the cases of clinical and histological "activity" than in the latent group (Table X).

Miscellaneous diseases involving the liver. Results are recorded in Table X. It is seen that the investigation of serum cholesterol gives little additional diagnostic information in this group.

Use of the serum cholesterol as a guide to the severity of liver damage.

TABLE XI
THE SERUM CHOLESTEROL CORRELATED WITH THE HISTOLOGICAL SEVERITY OF ACUTE HEPATITIS.

Histological grade	Mean	S.E. Mean	Range	Range of values in mg/100ml.						Totals
				110	140	170	200	230	260	
				to	to	to	to	to	to	
				139	169	199	229	259	289	
A	198	11.6	285-111	2	3	4	3	6	1	19
B	194	10.4	266-124	1	7	3	6	4	1	22
C	185	10.6	250-120	1	2	3	2	0	0	8
D	175	7.5	217-110	1	0	2	1	0	0	4

Acute hepatitis. Although the blood level of cholesterol esters may be useful in assessing the severity of a liver lesion, it is generally agreed that total blood cholesterol gives little assistance in this respect. We have found no significant difference between the mean serum cholesterol for the four histological grades of hepatitis (Table XI). The estimation was of no value in foretelling either the duration of illness or the eventual prognosis.



Obstructive jaundice. ⁵² It has been suggested that the progressive liver cell changes occurring in the course of obstructive jaundice might be reflected in a diminishing blood cholesterol. (Adler and Lemmel, 1928; Epstein, 1932; Stroebe, 1932). This was not confirmed by Epstein and Greenspan (1936) who recorded a fall only as terminal cholaemia developed. In the series reported in this paper the cholesterol showed no fall as the duration of obstruction increased. Total cholesterol readings cannot be used to estimate the degree of secondary liver damage in obstructive jaundice.

Cirrhosis of the liver. As mentioned above, there was no difference in the total serum cholesterol between the cases of cirrhosis in which cell necrosis was demonstrated and those in which it was absent. The cases which terminated fatally did not show significantly different serum cholesterol readings from those in which improvement occurred, and discharge from hospital was possible.

CHAPTER VIISERUM PROTEINS

Serum protein estimations in the investigation of liver diseases have many of the drawbacks already referred to in the discussion of serum cholesterol. It is noted that the range of values in normal subjects is very wide.

Most of the evidence suggests that the liver is solely responsible for the synthesis of serum albumen. Serum globulin may partly originate in the cells of the reticulo-endothelial system (Sabin, 1939). In liver diseases the main defect is in the manufacture of serum albumen. In some liver diseases, particularly those of a chronic nature, there is an apparently partially "compensatory" increase in serum globulin. The common changes in the serum proteins produced by liver diseases are, therefore, hypoalbuminaemia, hyperglobulinaemia and a consequent diminution in the albumin/globulin ratio (Madden and Whipple, 1940; Post and Patek, 1942). Electrophoretic analysis has shown the gamma globulin component to be mainly affected in the increased globulin (Longsworth, Shedlovsky and MacInnes, 1939; Luetscher, 1940; and Gray and Barron, 1943). The changes in serum globulin are believed to be responsible for the occurrence of such precipitation tests as the Takata-Ara reaction, the cephalin-cholesterol flocculation test (Hanger, 1939), the colloidal gold reaction (Gray, 1940; MacLagan, 1944b), and the thymol blue turbidity test (MacLagan, 1944c). Changes in the serum proteins can be produced by many conditions which disturb the normal nitrogen balance

Of the body. Such factors as malnutrition, alimentary tract disorders, fever or haemorrhage, may per se lead to hypoproteinaemia and, if occurring in conjunction with liver disease, may lead to wrong interpretation of serum protein findings. The various causes and results of hypoproteinaemia have recently been reviewed (Madden and Whipple, 1940; Elman and Lischer, 1943; Wilensky, 1944). Scrutiny of Table XII will show that serum protein estimations have little place in the differential diagnosis of liver diseases. The findings in this series will therefore be discussed mainly in relation to their value in estimation of the extent of the liver damage.

Cirrhosis of the liver. The most marked changes in the serum proteins occur in cirrhosis of the liver, and this condition will be considered first. Alterations in the serum proteins in this disease have been described for about forty years. Total serum protein decreases have been recorded by Grenet (1907), Gilbert and Chiray (1907), Abrami and Wallich (1929) and others. It was later noted that the diminution was due particularly to a fall in serum albumen (Myers and Keefer, 1935; Foley, Keeton, Kendrick and Darling, 1937). Tumen and Bockus (1937) believe that the hypoalbuminaemia is a much more significant and constant finding in liver disease than the elevation of the serum globulin or the lowering of the A/G ratio. The protein changes occur in a high proportion of cirrhotics. Cates (1941) records 15 out of 25 cases with low total serum protein and reversal of the A/G ration. Gottardo and Winters (1942) record profound serum protein

changes in 100 per cent of their 24 cases of portal cirrhosis; a fall in serum albumen was the most constant finding. Post and Patek (1942), as a result of their very thorough investigation of 61 cases, recorded an abnormal A/G ratio in 54 of them. These authors stress the value of the serum albumen level in determining prognosis.

In the present series, the serum proteins were observed in 8 cases of "inactive" liver cirrhosis. 7 of these were normal; in the eighth there was a very slight increase in globulin with a low A/G ratio. This latter was not reversed. In inactive cirrhosis, therefore, the serum proteins are usually normal. A very different state of affairs was encountered in the 12 cases of "active" cirrhosis. All except one case showed a normal total serum protein level. (This one exception was a very low level in a man who had had a severe haematemesis 48 hours previously.) In the other cases the components of the serum protein had been greatly altered. Of the 11 cases, 9 had a serum albumen of less than 3.4 g. per 100 ml. and in 7 of them the level was below 3 g. per 100 ml. The disease proved fatal in these latter 7 cases, so that serum albumen determinations are of great prognostic value. The serum globulin in 10 of the 11 was greater than 3 g. per 100 ml. and in 8 the value was greater than 4 g. per 100 ml. There was a resultant change in the A/G ratio. In all 12 cases the ratio was less than 1.3 and in 10 cases there was reversal of the A/G ratio. In active cirrhosis, therefore, pronounced serum protein changes occur constantly. These changes do not serve to differentiate cirrhosis from other

TABLE XII

*
THE TOTAL AND DIFFERENTIAL SERUM PROTEINS
IN 97 CASES OF LIVER DISEASE.

Diagnosis	No. of cases	Total serum proteins g./100 ml.			Serum albumen g./100 ml.			Globulin g/100 ml.		
		Mean	S.E. Mean	Range	Mean	S.E. Mean	Range	Mean	S.E. Mean	Range
Acute hepatitis	41	6.04	0.7	8.1-4.5	3.5	0.12	4.7-2.1	2.54	0.13	4.7-1.0
Obstructive jaundice	12	6.02	0.2	8.3-3.9	3.3	0.18	4.7-1.9	2.73	0.14	3.6-2.0
Cirrhosis										
Active	12	6.8	0.36	9.0-4.6	2.8	0.09	3.7-2.0	4.0	0.28	5.5-2.0
Latent	8	6.7	0.42	7.2-5.9	4.2	0.25	4.8-3.5	2.5	0.14	3.3-2.0
Haemolytic jaundice	5	6.4		7.9-5.4	4.2		4.9-3.3	2.2		3.2-1.5
Misc. blood diseases	3	6.5			3.8			2.7		
Hydatid cyst	4	6.3		6.8-5.8	3.7		3.8-3.6	2.6		3.0-2.2
Amoebic abscess	1	6.0			3.9			2.1		
Weill's disease	1	4.8			2.4			2.4		
Kala Azar	3	6.9		7.0-6.0	4.4		4.6-4.1	2.5		2.3-1.4
Amyloid disease	2	5.8			2.8			3.0		
Secondary malignant disease	5	5.5		6.1-5.1	3.5		4.4-2.4	2.0		3.2-1.0

*

Normal total serum protein 6-8 g./100 ml.

Normal serum albumen 3.4-6.0 g./100 ml.

Normal serum globulin 1.5-3.0 g./100 ml.

A/G ratio 1.3-4.

THE SERUM PROTEINS BEFORE AND AFTER RECOVERY

IN 21 CASES OF ACUTE HEPATITIS.

	Mean duration of jaundice (days)	Total serum proteins g./100ml.		Serum albumen g./100 ml.		Serum globulin g./100 ml.		A/G Ratio	
		Mean	S.E. Mean	Mean	S.E. Mean	Mean	S.E. Mean	Mean	S.E. Mean
Acute stage	12	6.02	0.17	3.41	0.14	2.6	0.11	1.31	0.11
After recovery	32	6.51	0.14	4.25	0.15	2.25	0.12	1.9	0.16

TABLE XIV

THE TOTAL AND DIFFERENTIAL SERUM PROTEINS CORRELATED

WITH THE HISTOLOGICAL SEVERITY OF ACUTE HEPATITIS.

Histological grade	No. of cases	Total serum proteins g./100 ml.			Serum albumen g./100 ml.			Serum globulin g./100 ml.		
		Mean	S.E. Mean	Range	Mean	S.E. Mean	Range	Mean	S.E. Mean	Range
A	12	6.4	0.4	8.1-5.2	3.7	0.03	4.6-3.1	2.7	0.2	4.7-1.0
B	19	6.1	0.15	7.1-4.8	3.8	0.14	4.7-2.1	2.3	0.2	3.6-1.0
C	6	5.6	0.21	6.2-4.5	3.0	0.27	3.8-2.4	2.6	0.05	3.0-2.1
D	4	5.8	0.3	6.8-5.0	2.6	0.14	3.0-2.0	3.2	0.45	4.0-2.4

forms of liver disease ⁵⁸ in which identical changes occur, although not usually so constantly or to such a pronounced degree.

Acute hepatitis. Changes in the serum proteins, of a similar type but to a lesser extent than those encountered in cirrhosis, are reported in acute hepatitis (Filinski, 1922; Tumen and Bockus, 1937; Turner et al., 1944). Higgins et al. (1944) mentioned that the serum albumen is particularly diminished in those cases of greater than two months' duration and the albumen is then roughly proportional to the degree of liver damage. As a result of observations made during the North African epidemic, the United States' instruction on jaundice (1944) states that the plasma albumen is usually normal in acute hepatitis, an increase in globulin being the characteristic finding. Lucké (1944) studied fatal cases of hepatitis. He mentions another difficulty in the interpretation of plasma proteins, namely that therapeutic plasma infusions lead to a false raising of the blood proteins; hence the usual fall in plasma albumen preceding death was sometimes masked. The findings in our series are shown in Table XII. The mean total serum protein for 41 cases is 6.04, that is, about the lower limit of normal. The serum albumen, serum globulin and the A/G ratio also fell within the normal range. That there has, in fact, been a divergence from the normal for that particular group is revealed when the serum proteins at the height of the disease are compared with those existing after recovery. This was done in 21 cases (Table XIII). After recovery, the total serum protein did not significantly alter; there was,

59

however, a rise in serum albumen, a fall in serum globulin, and a rise in the A/G ratio. All these latter findings are statistically significant and occurred within the wide normal range of values. Hence, in acute hepatitis there is a change in the serum proteins in a similar direction to that found in cirrhosis. It is not usually sufficiently conspicuous to be recognised or to be of diagnostic importance. It is unmasked when follow-up studies after recovery are made.

Comparison of the serum proteins in the various histological grades of hepatitis shows a significant difference between the less severe (A and B) and the more severe (C and D) grades, (Table 14). In the more severe grades the total serum protein level was lower and the serum albumen was more reduced. The cases in the group of maximal severity (D) showed significant hyperglobulinaemia and a reversal of the A/G ratio. The only 3 fatal cases were in this group, and all had serum albumen levels of 3 g. per 100 ml. or less. Apart from one case, a very undernourished old lady, levels of serum albumen of 3 g. or less were encountered only in the severe histological grades of hepatitis. Such a reading is useful in detecting the histologically severe case. The total serum protein level, the serum globulin and the A/G ratio were of less value in assessing severity.

Obstructive jaundice. The chief change in the serum proteins reported in obstructive jaundice is hypoalbuminaemia (Tumen and Bockus, 1937; Higgins et al., (1944)). Obstructive jaundice, however, is associated with digestive disturbances and often

malignant disease. These latter conditions can themselves cause cachexia and hypoproteinaemia (Peters and Eisenmann, 1933). This partly explains the difficulty in correlating the serum protein changes with the liver cell damage resulting from the biliary obstruction. However, this lessens the use of the estimation in the distinction of obstructive from primary parenchymatous jaundice.

Our results in 12 cases (Table XII) agree with those of other workers. The mean total serum protein is low. This is attributed to the fall in albumen, the globulin being almost unaltered. 7 of the 12 cases had a serum albumen of 3 g. per 100 ml. or less. The total serum protein and the serum albumen diminished with increasing duration of obstruction and cachexia.

GALACTOSE TOLERANCE AND THE LIVER

Ever since 1857, when Claud Bernard established the glycogenic function of the liver, the attention of investigators has been focussed on the use of carbohydrate tolerance tests to assess liver function. Many difficulties have been encountered. A great number of factors other than the liver are concerned in sugar metabolism. Variations in intestinal absorption, in tissue utilisation or in endocrine influences make interpretation of the test results very difficult. In the selection of a carbohydrate to be used as a test of liver function three criteria should be observed.

1. The carbohydrate should be metabolised only by the liver.
2. The metabolism should be independent of endocrine influences and especially of insulin.
3. The metabolic process should be difficult so that as great a load as possible is placed on the hepatic cell.

Glucose is clearly quite unsuitable. It is metabolised not only by the liver but also by every tissue in the body. Glucose metabolism is linked up with the secretions of almost all the endocrine glands. The liver finds its metabolism fairly easy.

The laevulose tolerance test was introduced by Strauss in 1901. Comprehensive reviews of its uses in testing liver function have been made by Stewart, Scarborough & Davidson (1938) and Herbert and Davison (1938). The galactose tolerance test appeared five years later (Bauer, 1906), and it is galactose

tolerance tests which will be mainly considered in this thesis.

The liver is probably the only organ capable of utilising galactose. Draudt (1913) and Fischer (1925) showed that after administration of galactose to dogs with Eck fistulae 80-85% was excreted in the urine. Bollman, Mann and Power (1931) found that galactose administered intravenously to normal dogs disappeared from the blood in two hours. 10-30 per cent was recovered from the urine. In a similar experiment on hepatectomised animals the galactose also disappeared from the blood, but now 50-60 per cent appeared in the urine. There was no rise in blood glucose. Conversion of the galactose to glucose was therefore unlikely. Mann & Magath (1922) state that galactose would not relieve the hypoglycaemia of hepatectomised dogs. Roe, Gilman & Cowgill (1935) administered galactose to dogs and then measured the arterial and venous galactose contents of the blood circulating to muscles, brain and liver. Only in the last mentioned organ could an arterio-venous galactose difference be detected. Foster (1923) measured the tissue arterio-venous sugar difference after administration of glucose, fructose or galactose. Galactose showed no arterio-venous difference. Tissue utilisation of galactose, therefore, did not occur. The tissues were able to assimilate laevulose or glucose. The inability of muscle to metabolise galactose has been demonstrated by various workers (Laquer and Meyer (1923); Griesbach (1929); Arcq (1931)). Wörner (1922) found that galactose tolerance was the same whether the sugar was given by mouth or injected into the portal veins. The evidence that laevulose is metabolised

solely by the liver rests on a less secure basis.

Bollman and Mann (1921) found that, in the hepatectomised animal, fructose could be readily converted to glucose. The disappearance of ingested laevulose was not very dissimilar to that occurring in the normal animal. Enterectomy prevented the conversion. This suggested that the intestines could convert fructose to glucose. Verzáar and MacDougall (1936) also believe that the intestinal mucosa can convert fructose to glucose. Goda (1938) found that the isolated kidney could convert laevulose to glucose. The work of Foster (quoted above) also suggests that the tissues can utilise laevulose.

The metabolism of galactose is independent of insulin. Roe and Schwartzmann (1932) found essentially the same galactose tolerance in diabetics as in normal subjects. In rabbits, insulin did not accelerate the disappearance of galactose from the blood. Shay, Schloss & Rodis (1931) found that, after oral administration of galactose to normal subjects, insulin did not alter the degree of galactosuria. We have performed galactose tolerance tests on three uncontrolled diabetics with normal results. There is evidence that laevulose also is metabolised independently of insulin. (Wierzuchowski, 1926; Copley, 1929; Bollman & Mann, 1931; Davidson, 1936).

The effect of the thyroid gland on the intestinal absorption of galactose will be dealt with later.

The liver probable finds it more difficult to deal with galactose than with other sugars. Deuel, Gulick & Butts (1933), in rats, measured the ability of the liver to form glycogen from different carbohydrate

sources. After four hours' glucose or fructose absorption the liver had formed 4-5% glycogen, whereas after six hours' galactose absorption only 1.26% glycogen had been produced. Foster, (1923) found that oral administration of 40-100g. galactose to normal adults caused hyperglycaemia. Dosage with fructose failed to cause any marked increase in the blood sugar. Bodansky (1923) found that dogs had a better tolerance for dextrose than for galactose. Folin and Berglund (1922) state that the limits of assimilation for laevulose are so high that they can scarcely be exceeded. Isaac & Adler (1921) confirm this work. In metabolising galactose, therefore, a maximum strain is placed on the glycogenic functions of the liver.

The basic evidence for the use of galactose as a suitable test substance for liver function has been given. There are strong experimental grounds to support its use. We now consider whether animals having experimentally produced liver damage do, in fact, show impaired tolerance for galactose. Roubitschek (1912) showed that marked galactosuria occurred after experimental phosphorus poisoning. Improvement in galactose tolerance coincided with liver cell regeneration. King et al. (1940) demonstrated impaired galactose tolerance in rabbits following poisoning with carbon tetrachloride. Bollman, Mann & Power (1935), using carbon tetrachloride, produced acute degenerative changes in the livers of dogs. These animals showed impaired galactose tolerance. However, extensive experimental cirrhosis of excision of 70-80% of the liver failed to produce increased

galactosuria. It therefore appears that liver damage must be of a very extensive and acute nature before an interference with the powers of the organ to metabolise galactose is produced. This will be corroborated by the clinical observations.

The original galactose tolerance test of Bauer consists of the oral administration of 40 grams of galactose. The galactose excretion in the urine is then measured; 3 grams in five hours is the upper limit in normal subjects. This technique at once introduces a number of factors unrelated to the liver. Galactose given by mouth may not be adequately absorbed from the alimentary tract. Gastrostasis or vomiting, intestinal hurry and diarrhoea interfere with absorption. Banks et al. (1933) aspirated the stomachs of their patients at the end of the five hour period. Variable amounts of unabsorbed galactose could be recovered. Davies (1927) believes that ascites with portal stagnation delays absorption of the galactose.

The rate of absorption of galactose from the intestines also depends on extra hepatic factors. Probably the most important is the thyroid gland. Thyrotoxicosis is associated with impaired tolerance to oral galactose (Lichtman, 1932; Althausen and Weyver, 1937). Intravenous galactose is metabolised normally. The defect in thyrotoxicosis is therefore not in the liver but in an increased absorption of the sugar. (Althausen et al., 1940 & 1941; Barnes and King, 1943). The oral galactose test is used to measure the degree of thyrotoxicosis. (Althausen et al., 1940; MacLagan and Rundle, 1940; Barnes and King, 1943).

Althausen and his co-workers (1940) believe that the oral galactose test is a more sensitive index of thyrotoxicosis than is the basal metabolic rate. Difficulties in intestinal absorption can be circumvented by giving the galactose intravenously. (Pollak, 1932; Budak, 1933; Jankelson et al., 1936; King and Aitken, 1940; Bassett et al., 1941).

The kidney is another extrahepatic factor entering into the oral test. The functional state of the kidney might influence its ability to excrete galactose. It is usually stated that galactose has no renal threshold. (Folin and Berglund (1922); Rowe and Chandler (1924); Harding et al. (1934); Bassett et al. (1941)). Goldblatt (1925) showed that oral administration of galactose to normal subjects was followed by excretion of the sugar in the urine; simultaneous estimations of the blood sugar revealed no rise. A renal threshold for galactose was therefore considered unlikely. Roe and Schwartzmann (1933), however, found that under similar conditions the urinary excretion did not parallel the blood galactose levels. A renal threshold for galactose was postulated. This was considered to vary in different subjects. Davies (1927) also found the galactose renal threshold to be most indefinite, even in normal subjects. Meczner (1933) noted that impairment of urinary galactose excretion occurred in renal diseases. The oral galactose test could be used, not only as a liver function test, but alternatively to assess renal function. If oliguria occurs during the test period, then a false result is

recorded. (Schiff and Senior, 1934). The difficulty of collecting complete correctly timed urinary samples from sick people without resorting to the catheter also adds an inaccuracy to the method. The test could therefore be improved by making the technique independent of urine analysis. The earliest effort to circumvent the kidneys was the measurement of blood sugar instead of urinary galactose (Pollak (1932); Budak (1933)). The rise in blood sugar which occurs after oral or intravenous galactose is not necessarily due to galactose. If extrahepatic factors cause simultaneous fluctuations in the blood sugar, then the results will be unreliable. The development of a specific method for estimating blood galactose (Raymond and Blanco (1928); Grant and Glaister (1933); Harding and Grant (1933)) led to the galactose tolerance test being put on a more satisfactory basis.

The methods by which galactose tolerance may be used as an index of liver function have been analysed. The most satisfactory technique is probably the intravenous administration of galactose followed by interval estimations of the blood galactose levels. This has been the procedure in our series.

Use of the intravenous galactose test in the differential diagnosis of liver disease.

Acute hepatitis and obstructive jaundice. Wörner, surveying the literature up to 1919, presented strong evidence that the oral galactose tolerance test was helpful in the diagnosis of acute parenchymatous from obstructive jaundice. This was confirmed by later workers (Shay et al., 1931; Owen, 1934). Other reports minimised the diagnostic value of the test

(Piersol and Rothman, 1928; Banks ⁶⁸et al., 1933).

With the introduction of the intravenous test, conclusions drawn were more uniformly favourable. Bassett et al. (1941) found that 81 per cent of their cases of parenchymatous jaundice gave a positive result and 82 per cent of obstructive jaundice cases of less than six months' duration were negative. King and Aitken (1940) state that in nearly all cases the intravenous test distinguished clearly between jaundice due to liver cell damage and jaundice due to gross obstruction of the biliary tract without liver damage. MacLagan (1944a) states that the test is of some value in distinguishing the groups of jaundice if interpreted with due regard to clinical data.

We were unable to confirm these favourable reports. In 15 out of 49 cases of histologically confirmed acute hepatitis the galactose time was less than the mean value for normal subjects. In 15 out of 18 cases of obstructive jaundice the galactose time was greater than the mean normal value. It is evident, therefore, that acute hepatitis often occurs in the presence of normal galactose tolerance, and obstructive jaundice may be associated with impaired tolerance. The overlap between the two groups is well shown in Table 15 on next page, and it seems that the galactose tolerance test is of no value in the diagnosis of obstructive from parenchymatous jaundice.

Cirrhosis of the liver. Results obtained with the galactose tolerance test in hepatic cirrhosis are very variable. Pollak (1932) found impaired tolerance in only 10-36 per cent of his series, whereas Bassett et al. (1941) found 31 out of 32 cases of cirrhosis

TABLE XV.

THE GALACTOSE TIME IN 100 CASES OF LIVER DISEASE.

Diagnosis	Galactose time(mins.)		Range of values of galactose time in minutes.					Total
	Mean	Range	0-39	40-59	60-79	80-99	Greater than 100	
Acute hepatitis	82	184-30	7	6	14	5	17	49
Obstructive jaundice.	89	158-30	3	0	4	4	7	18
Cirrhosis								
Active	106	134-69	0	0	1	3	6	10
Latent	52	72-44	2	3	2	0	0	7
Haemolytic jaundice.	45	51-35	1	3	0	0	0	4
Hydatid cyst	67		0	0	2	0	0	2
Amoebic abscess	39		1	0	0	0	0	1
Weil's disease	30		1	0	0	0	0	1
Kala Azar	31		3	0	0	0	0	3
Amyloid disease	65		0	0	2	0	0	2
Secondary malignant disease.	56		1	0	2	0	0	3

Normal mean galactose time 61 minutes (range 30 - 92 minutes).

had impaired galactose tolerance. Other workers mention the variability of galactose tolerance in cirrhosis (Davies, 1927; Schiff and Senior, 1934; Jankelson, Segal and Aisner, 1936). This variability may be due to lack of distinction being made between the acute "active" and the chronic "latent" cases. Banks et al. (1933) made such a distinction and found normal galactose tolerance except in the acute episodes with jaundice. This agrees with our findings; 9 of the 10 cases of cirrhosis showing the histological evidence of activity gave markedly abnormal galactose tolerance tests. Of the 7 cases of latent compensated cirrhosis only one gave an abnormal galactose tolerance test.

A diagnosis of active cirrhosis with jaundice should not be made if the galactose tolerance is normal. The results in our series suggest that hepatic cirrhosis cannot be diagnosed from other forms of jaundice on the basis of the galactose tolerance test.

Miscellaneous diseases involving the liver. Cases covering a wide range of diseases with liver involvement have been studied. These include amyloid disease, amoebic liver abscess, hydatid cysts, malignant deposits not causing jaundice, and cases of haemolytic jaundice of varying aetiology. In all these cases the galactose tolerance was normal (Table 15 on next page). Apart from one case of cirrhosis, an abnormal galactose test has not been encountered in the absence of jaundice. It seems of little help to perform the galactose tolerance test

for the diagnosis of probable liver disease in the absence of jaundice.

Use of the intravenous galactose tolerance test in the determination of the extent of liver cell damage.

Acute hepatitis. The fact that in many cases of acute hepatitis galactose tolerance is normal has already been mentioned. Analysis of the cases into the varying degrees of histological severity shows that, with a few exception, the cases with normal tolerance fall into the grade with the least degree of liver cell damage (Table 16). TABLE 16.

THE GALACTOSE TIME CORRELATED WITH THE HISTOLOGICAL SEVERITY OF ACUTE HEPATITIS

HISTOLOGICAL GRADE	Galactose time (mins)		Range of values of galactose time in mins.					Total
	Mean	Range	0-39	40-59	60-79	80-99	Greater than 100	
A	55	68-30	3	6	6	0	0	15
B	86	127-35	3	0	8	2	11	24
C	113	184-30	1	0	0	2	5	8
D	101	104-97	0	0	0	1	1	2

This lack of sensitivity of the galactose test to the lesser degrees of liver damage has been noticed by other workers (Drill and Ivy, 1944). We have observed a progressive increase of galactose time with increasing histological severity of hepatitis. Galactose tolerance, therefore, probably does truly reflect the state of the liver. The normal tolerance in many cases of lesser severity means that a severe degree of acute diffuse liver damage is essential before tolerance is impaired. This may be explained by the histological picture of acute hepatitis. The surviving liver cells are seen

to retain their complement of glycogen. This suggests that these cells retain their power of metabolising glycogen. It is not surprising, therefore, that tests of the carbohydrate functions of the liver run in parallel with the percentage of surviving liver cells. Moreover, because of the great reserve power of the liver, the cases of minimal histological severity will show little or no impairment of galactose tolerance. Jankelson et al. (1936) mention that the degree of retention of galactose in the blood is greatest at the height of disease and is negative in subsiding infective hepatitis. Tumen and Piersol (1933) also state that the last is normal if employed late in the course of the illness. Our results are in keeping with these findings. The relation between the duration of the jaundice and the results of the galactose tolerance test are shown in Fig.17 on following page.

Impaired tolerance is encountered more often in the first 14 days than later in the disease. This is in keeping with the recognised clinical and pathological course of acute hepatitis. The liver damage probably starts before the onset of icterus, and very rapidly reaches a maximum. After two weeks' jaundice, the patient is usually well on towards recovery.

Therefore, the earlier the case is studied, the more likely is galactose tolerance to be abnormal. In 13 cases in which the galactose tolerance test was initially abnormal, the test was repeated later in the course of the disease. The results are seen in Table 17. All had returned to normal in an average of 13 days after the first test. In 10 cases, despite

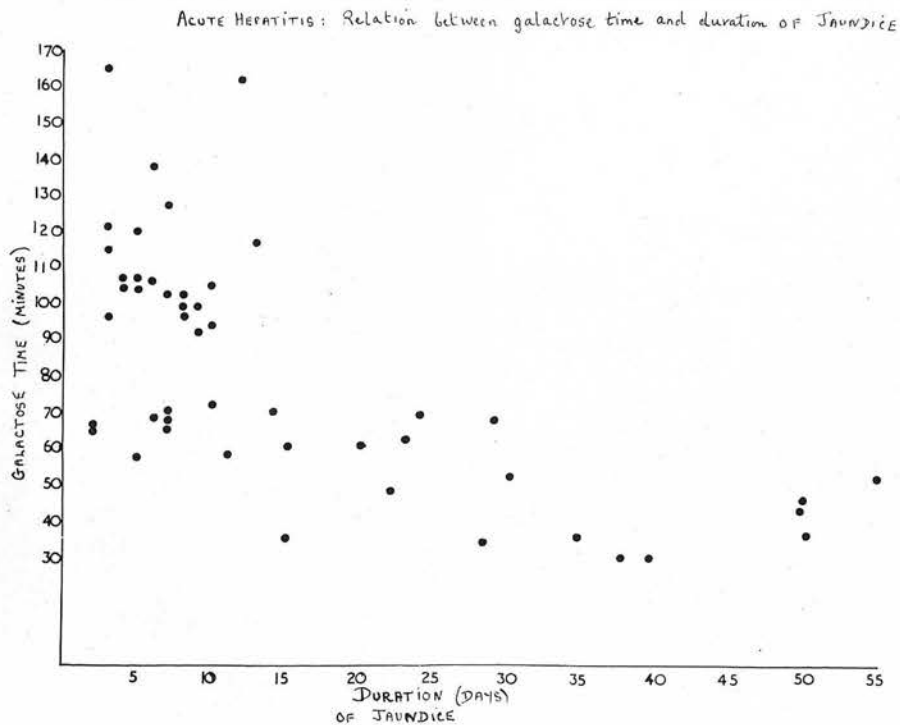


TABLE XVII

ACUTE HEPATITIS: TIME TAKEN FOR RESTORATION OF NORMAL
GALACTOSE TOLERANCE.

Case	Sex	Age	Histol- ogical Grade	Impaired Galactose tolerance			Normal galactose tolerance		
				Duration of jaundice (days)	Serum Bilirubin mg/100.ml	Galactose time (minutes)	Duration of jaundice (days)	Serum Bilirubin mg./100ml.	Galactose time (Minutes)
1	M	37	B	3	4.4	96	18	2.1	45
2	M	27	B	8	8.4	97	28	17.0	71
3	M	21	B	7	3.5	102	23	2.5	68
4	M	58	B	4	2.5	104	19	0.5	70
5	M	41	B	6	8.0	106	11	2.9	76
6	M	34	B	7	5.4	127	19	1.2	63
7	M	34	B	14	3.4	117	31	1.1	65
8	M	41	C	9	11.2	92	17	3.8	40
9	M	28	C	10	11.5	94	18	6.0	53
10	M	38	C	10	18.0	105	17	3.3	73
11	F	20	C	12	7.1	162	24	6.5	61
12	M	45	C	3	8.4	151	30	2.9	63
13	M	61	D	7	18.0	97	18	14.0	67
<u>Mean</u>				8	8.4	112	21	4.9	63

acute hepatitis, particularly of the less severe type. Recovery of galactose metabolising power does not indicate complete recovery of normal liver structure. The prognostic use of the test is therefore minimal.

Cirrhosis of the liver. As we have seen, acute diffuse liver cell damage is essential for impairment of tolerance for intravenously administered galactose. It is not surprising, therefore, that cases of inactive or latent cirrhosis nearly always have normal galactose tolerance. In this condition, the liver cells usually appear normal and the presence of fibrous bands in the liver does not seem to alter galactose metabolising function.

A very different state of affairs exists when the cirrhosis is in an active "decompensated" state. In these cases there are cell necrosis throughout the liver. The regenerating nodules of liver cells cannot compensate for the diffuse liver damage. Impairment of galactose tolerance was therefore almost constantly found in this group.

Obstructive jaundice. Changes in the liver of an acute diffuse nature follow rapidly on obstruction to the common bile duct. It is therefore not surprising that the galactose tolerance test is abnormal in this condition. If the obstructive jaundice is associated with biliary cirrhosis or massive hepatic malignant deposits, the impairment of galactose tolerance is particularly conspicuous. It has been reported that galactose tolerance becomes more impaired as the duration of obstruction increases. In this small series of 18 cases no definite correlation could be established between the galactose and the duration of icterus. 5 cases of obstruction, which were not

surgically relieved, and which showed initially normal galactose time, were followed through the course of the illness. The galactose tolerance of all eventually became impaired. There seems little relation between the level of the serum bilirubin and the galactose time. The test may be useful as a pre-operative measure, a positive result indicating the associated liver damage.

Miscellaneous liver diseases. Acute diffuse liver cell damage is essential for impairment of galactose tolerance. Therefore localised lesions such as abscesses, cysts and malignant deposits without jaundice are not detected and their severity cannot be estimated by a galactose tolerance test. Similarly haematological disorders, in which the main change in the liver is an infiltration with abnormal blood cells, and in which the liver cell columns remain intact, are not associated with a raised galactose time.

HIPPURIC ACID SYNTHESIS

All the known activities of the liver have, at one time or another, been adapted for use as a measure of hepatic function. The detoxicating power has proved no exception, and various toxic agents have been tested as substances with which to assess liver function. Most of them, such as cincophen, proved too harmful for general application. Benzoic acid is detoxicated by conjugation with glycine to form hippuric acid. Benzoic acid is relatively innocuous and Hippuric acid synthesis soon found a permanent place in the array of tests at the disposal of the clinician anxious to have some guide to the working of the liver. The specificity of the test for hepatic as opposed to renal function is not yet certain, and the relative evidence at present available, together with our own observations will be discussed.

The synthesis of hippuric acid from benzoic acid and glycine was discovered by Wöhler in 1824. Since then the mechanism of synthesis and its site of origin in man and animals has been much investigated. Confusion has arisen because both the kidneys and the liver appear to take part in the metabolism of hippuric acid. There is a marked species variation; in some animals, for example the dog, the kidney is the main site of formation, whereas in man the liver apparently plays the more important part. Attempts have been made to establish hippuric acid synthesis as a kidney test,

and it was later, when the role of the liver in its production was realised, that it was adapted as a test of hepatic function.

The technique employed for the intravenous hippuric acid test has been out-lined previously. Difficulties occurred both in the analysis and in the interpretation of results. These will be discussed first.

Method:

The intravenous technique of Quick, Ottenstein and Weltchek (1938) was used. The patient is allowed a light breakfast. The sodium benzoate is made up in an 8.85 per cent solution; 20 ml. of this containing 1.77 g. sodium benzoate and equivalent to 1.5 g. benzoic acid, are injected intravenously. The injection should take at least five minutes. Immediately after completion of the injection and again one hour later the bladder is emptied. The whole one-hour specimen of urine is sent to the laboratory. An adequate urine specimen is ensured by giving the patient one pint of water to drink just before injection.

Toxicity of Sodium Benzoate.

Quick (1931) has classified the toxic effects.

1. Local irritation to the gastro-intestinal mucosa:-
Nausea, vomiting, anorexia and diarrhoea.
2. Central nervous system:- Headache, tinnitus, vertigo, giddiness and reflex vomiting.

We have found transient toxic effects common. The most usual are giddiness, blurring of vision and lights in front of the eyes. A peculiar taste is often experienced and some patients complained of

abdominal colic. Using the intravenous test, no subject vomited; a high proportion of the few patients who took the benzoate by mouth were troubled with this symptom. The unpleasant effects pass off within three minutes and have never caused prolonged discomfort or given rise to serious complaints. Slow injection lessens the incidence of side effects.

The reported toxic dose of sodium benzoate varies greatly with different authorities Meissner & Shepherd (1866) state that 5.7 g. produces gastrointestinal symptoms. Wiley (1908) quotes as little as 0.9 - 2.5 g. by mouth as producing nausea and vomiting. Chittenden et al. (1909), however, report no ill effects from up to 4 g. and Bignami (1924) and Lewinski (1908) have given 25 - 40 g. with only minor symptoms. Bryan (1925) states that normal people can detoxicate 25 g. of sodium benzoate without untoward effects. Kinsey & Wright (1944) report severe reactions following the injection of sodium benzoate in a patient with extensive liver damage. These consisted of severe substernal pain, shock, increased icterus and granulocytopenia. The changes were attributed to inability of the diseased liver to detoxicate sodium benzoate to relatively non toxic substances. It seems unlikely however that the 1.77 g. given in the usual intravenous test will result in severe toxic symptoms.

Analysis:

The method is that of Weichselbaum and Probst (1938). The urine volume is measured. If the volume is large the specimen is acidified with acetic acid and boiled down to less than 200 ml. The urine is

now saturated with sodium chloride, 30 g. being added to each 100 ml. This is dissolved by heating and the urine filtered. The filtrate is made acid to congo red with 50 per cent sulphuric acid and an excess of 1 ml. added. The precipitation of hippuric acid is hastened by scratching the side of the vessel with a glass rod and if necessary by the addition of a crystal of hippuric acid. The flask is left over-night in the ice-box. Next day the hippuric acid crystals are filtered off with suction and are washed with 20 ml. 30 per cent sodium chloride solution. They are then transferred to a flask. On warming the crystals dissolve readily in distilled water. The solution is titrated against 0.5 N sodium hydroxide using phenolphthalein as an indicator.

Calculation.

Allowance is made for the 0.1 g. hippuric acid remaining in every 100 ml. of urine saturated with sodium chloride and for the hippuric acid dissolved by the 20 ml. saline wash. Weight of hippuric acid excreted (g) = number of ml. 0.5 N NaOH x 0.072 + 0.1 x $\frac{\text{volume of urine (ml.)} + 20 \text{ ml.}}{100}$

Analytical difficulties.

In some cases the hippuric acid fails to precipitate from the urine even after saturation with salt and acidification. The difficulty has received little emphasis in the literature. We have encountered it fairly commonly; it occurred in 31 tests on 21 subjects out of a total of 180 tests performed during the past 18 months. It occurs

more often in the intravenous than in the oral test, perhaps because the quantity of hippuric acid excreted is less. In 6 instances the failure coincided with impaired renal function (see later). In the other 15 the patient was markedly jaundiced and the urine was very dark in colour; 11 had obstructive jaundice and 4 acute hepatitis. The difficulty occurs most often when dealing with highly pigmented urines. The incidence of this difficulty in jaundiced subjects was 15 in 93 cases (16%). To obviate these difficulties the following precautions should be observed:-

1. Where a low excretion is expected the sample should be evaporated down to 50 ml. Less hippuric acid will then be held in solution and more become available for precipitation.

2. Dark urines are decolorized by boiling for a few minutes with Norit or charcoal. The specimen is then filtered (Weichselbaum and Probststein, 1938).

3. Any protein in the sample is removed by boiling in slightly acid solution followed by filtration.

4. After addition of the mineral acid the side of the vessel is scratched with a glass rod. A crystal or two of hippuric acid may be added to provide nuclei for crystallization.

5. The acidified specimen is left 24 hours in the ice chest.

6. As a last resort, a weighed amount of hippuric acid is dissolved in the urine by warming.

The total hippuric acid is estimated and by subtraction

the amount in the original sample determined.

Occasionally, despite these steps, precipitation does not occur. The quantity of substance may be too small or some interfering substance, possible a protective colloid, may be present in the urine.

Results in subjects without liver disease.

The intravenous hippuric acid test was carried out on 6 healthy persons and on 24 patients suffering from conditions unrelated to the liver. The hippuric acid is expressed in terms of sodium benzoate (Table 18).

TABLE 18.

THE INTRAVENOUS HIPPURIC ACID TEST IN
30 SUBJECTS NOT SUFFERING FROM LIVER DISEASES.

Age	Sex	Weight Kg.	Diagnosis	Urea clear- ance %	Volume Urine ml.	Excretion of hippuric acid. g. *
45	F	62	Normal	95	114	1.00
23	M	61	Normal	64	400	1.02
30	M	62	Normal	112	675	1.05
26	F	57	Normal	120	435	1.04
22	F	60	Normal	165	590	1.15
22	M	75	Normal	137	450	1.20
15	M	57	Convalescent from pneumonia	138	530	1.15
28	M	59	" " "	173	435	0.75
17	F	54	" " "	-	185	0.94
21	F	54	" " bronchitis	-	176	0.91
16	M	64	" " "	124	74	0.96
52	M	60	" " "	109	82	1.05
35	M	66	" " "	129	570	1.19
60	M	61	" " coron. thromb.	102	176	1.21
52	M	92	" " " "	99	335	1.21
44	M	66	Aortic stenosis, not in failure	130	435	0.95
32	M	61	Convalescent from tonsillitis	-	232	1.10
39	M	70	Osteoarthritis	148	70	1.13
59	M	61	Healed gastric ulcer	132	84	0.81
41	M	63	" " "	91	240	0.81
43	M	63	" " "	73	115	1.16
30	F	57	Convalescent from gastroenteritis	99	160	1.05
30	F	50	Hysteria	94	225	0.79
30	F	52	Vasovagal faints	80	340	0.88
55	M	62	Cerebral arteriosclerosis	57	323	0.88
43	F	40	Parkinson's disease	78	236	0.90
17	M	66	Vasovagal faints	130	70	0.93
50	M	60	Abducent neuritis	188	106	1.05
35	M	71	Meniere's syndrome	141	535	1.00
27	F	52	Neurosis	131	115	1.11

* The hippuric acid excretion in this and subsequent tables is expressed in terms of sodium benzoate.

The mean is 1.01 g., range 0.75 - 1.21.

S.D. is .088. These figures compare well with those quoted by Quick (1939). This author gives a range of 0.7 - 0.95 g. expressed as benzoic acid, which in terms of sodium benzoate becomes 0.83 - 1.12 g.

Relation of the Kidney to Hippuric Acid Synthesis.

In 1877, Bunge and Schmeideberg perfused the kidneys of dogs with benzoic acid and glycine. Hippuric acid was formed. Perfusion of other organs did not lead to hippuric acid production. These observations were repeated and verified 47 years later by Snapper, Grünbaum and Newberg. A further demonstration of the renal origin came from Bashford and Cramer (1902) who showed that the macerated renal tissue of the dog could synthesise hippuric acid in vitro. Borsook and Dubnoff (1940) gave similar reports on slices of kidney tissue examined in vitro, not only in dogs but also in rabbits, rats and guinea-pigs. Montes et al. (1942) showed that there was no change in hippuric acid synthesis in the rat after poisoning the liver with carbon tetrachloride. A renal origin for hippuric acid was postulated in this animal. The most pertinent observations on man were those of Kingsbury and Bell (1915). Two human kidneys were taken soon after surgical removal. These organs were perfused with blood containing sodium benzoate and glycine. Hippuric acid was found in the perfusing blood. Similar results were obtained with the kidneys of swine and sheep.

In man kidney disease is associated with impaired excretion of hippuric acid. (Jaarsveld and Stokvis (1879), Violle (1920)). Bryan (1925) used

the test as an index of kidney function. Kohlstaedt and Helmer (1936) recorded 9 cases of nephritis in which an impaired urea clearance occurred with a low hippuric acid test. Henderson and Splatt (1942) mentioned 11 similar instances. Even if the kidneys does not synthesize hippuric acid, its ultimate excretion demands adequate renal function. The power of excretion is 2.5 times the power of synthesis (Schwei and Quick, 1942), so that there is a wide margin for faulty kidneys. Kohlstaedt and Helmer (1936) and Moser, Rosenak and Hasterlik (1942) recommend that a simultaneous urea clearance should be run in parallel with the intravenous hippuric acid test. A one-hour urea clearance has been carried out in all our cases. Without a concurrent urea clearance, in 9 instances, diagnosis of liver disease based on the hippuric acid test would have been erroneous.
(see table 19 on next page.)

TABLE 19.

LOW HIPPURIC ACID EXCRETIONS ASSOCIATED WITH APPARENT
RENAL INSUFFICIENCY AND NORMAL LIVER HISTOLOGY.

Case	Age	Sex	Clinical Diagnosis	Liver Biopsy findings	Plasma Urea mg/100ml.	Urine Urea mg/100ml.	R.E.* %	Urine Vol. ml.	Hippuric Acid g.
1	68	F	? Ovarian tumour ? Cirrhosis of Liver.	Normal liver (confirmed operation)	163	2720	41	60	0.2
2	64	M	Jaundice 2 years ago. ? cirrhosis.	Normal liver histology.	89	1500	69	180	0.52
3	60	F	Haematemesis Post-transfus. jaundice.	Normal liver (conf. autopsy).	69	1800	90	190	0.3
4	45	M	Weil's disease.	Normal parenchyma. Residual portal tract scarring.	64	566	35	180	0.3
5	64	M	? Cirrhosis.	Normal liver.	32	1040	41	81	0.52
6	28	M	? Post-hepatitis cirrhosis.	Normal liver i. ii.	20 25	145 1100	27 156	170 240	0.36 0.74
7	26	M	? Post-hepatitis cirrhosis.	Normal liver i. ii.	17 22	72 765	48 128	510 166	0.52 1.2
8	26	M	? Cirrhosis ? jaundice	Normal liver (confirmed i. operation) ii.	32 37	375 725	31 174	120 400	0.7 1.16
9	31	M	? Cirrhosis	Normal i. Liver. ii.	43 47	282 445	53 92	365 435	0.4 0.92

*

R.E.% = renal efficiency as a percentage of the normal urea clearance.

Cases 1 to 4 were found to have raised plasma urea and the low hippuric acid excretion confirms the contention of Snapper and Grünbaum (1924) that azotaemia is associated with impaired hippuric acid excretion. In case 4 the nitrogen retention was due to the renal damage occurring in Weil's disease. The hippuric acid test should not be used as an indication of liver function in this condition.

Apart from actual defective kidneys, an apparently low urea clearance and hippuric acid test may be due to defective collection of the one-hour urine specimen. The bladder may not be completely emptied at the end of the test. Case 5 may be such an instance. Ideally the urine should be obtained by catheter before and at the end of the test period; this proves such an inconvenience that it has been avoided. In 4 instances (cases 6, 7, 8, 9 table 19) the test was repeated on the following day. On the second occasion the urea clearance was normal and the hippuric acid excretion more in keeping with the histological appearances of the liver as shown by biopsy. These cases were all young men and urine was passed in the upright posture. It is inconceivable that the remarkably low urea clearance could have been caused solely by retention of urine in the bladder at the end of the test period. The marked difference between the two renal function tests is the increase in the concentrating power of the kidney on the second occasion. It seems as if a transitory impairment of concentrating power can occasionally occur in otherwise normal kidneys and give rise to an abnormal urea clearance and a low hippuric acid excretion.

A urea clearance should be performed in conjunction with every intravenous hippuric acid test, not only to determine possible defects in renal function, but also as a check on faults in the urine collection. The two-hour consecutive urea clearance is more reliable than the one-hour. Wherever a low result for the hippuric acid test is associated with a low urea clearance, the test should be repeated with a urea clearance over two consecutive hours.

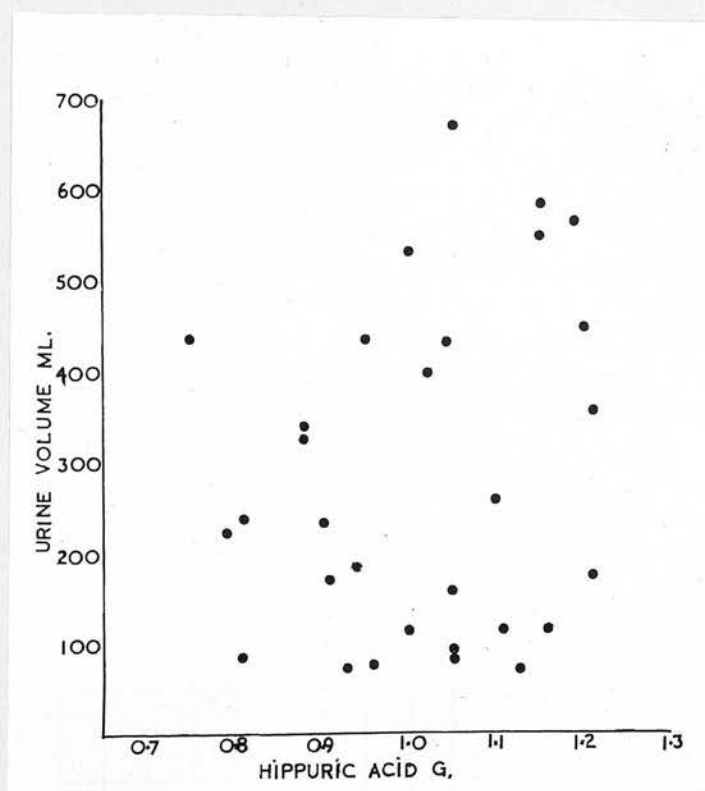
Relation of Hippuric Acid Excretion to the Volume of Urine.

Snapper and Grünbaum (1924) noted that patients with urea retention were able to eliminate more hippuric acid in a large volume of urine than in a small one. Machella, Helm and Chornock (1942) investigated 100 cases with hepatic dysfunction. A significant direct correlation existed between the amount of hippuric acid eliminated by the kidney and the volume of urine secreted during the same period. Higher than normal values were associated with large urine volumes and low values could be increased to normal by diuresis. In 4 normal controls and 8 subjects with liver disease the test was performed before and after water diuresis. A significant increase in hippuric acid excretion was produced in each case. Probststein and Londe (1940) diverge from these views. These authors found that in normal subjects no relation existed between urine volume and hippuric acid excretion. Boyce and McPetridge (1938) also state that if the concentrating power of the kidney is maintained, then low urine volumes are of little consequence. In view of these contradictory

opinions further experiments were carried out.

In 30 normal subjects the relation between the weight of hippuric acid synthesized and the volume of urine passed during the test period is shown. (Table 18 and Fig. 18).

18.



No correlation existed between the two factors.

(Correlation coefficient (r) is -0.0023).

In 10 normal subjects and 4 subjects with liver disease, in whom the output of urine was about 100 ml., the test was repeated after a water diuresis. The increased urinary output was ensured by giving

one pint of water 15 minutes prior to the injection and a further pint to be taken during the first half hour. Results are shown in Table 20 below.

TABLE 20

HIPPURIC ACID TEST PERFORMED WITH
LOW AND HIGH URINE VOLUMES

No.	Initials	Low volumes		Increased volumes	
		Urine vol. ml.	Excretion hippuric acid g.	Inc. volume ml.	Excretion hippuric acid g.
<u>Normals</u>					
1	V.W.	110	1.15	590	1.15
2	A.H.	66	1.01	450	1.20
3	J.H.	70	1.03	530	1.13
4	R.C.	132	1.03	650	1.02
5	S.S.	94	1.01	435	1.04
6	J.B.	94	1.14	570	1.00
7	A.N.	84	0.81	372	0.73
8	L.S.	50	0.73	435	0.75
9	A.H.	74	0.96	160	0.80
10	W.	46	0.70	82	1.05
<u>Liver Disease</u>					
1	A.G.	66	1.21	240	1.51
2	A.H.	125	0.69	900	0.73
3	J.T.	148	0.60	315	0.52
4	A.W.	60	0.31	400	0.33
	Mean	86	0.88	439	0.92

In these 14 instances, for a mean urinary volume of 36 ml. the mean hippuric acid excretion is 0.88 g. If the mean urine volume becomes 439 ml., the mean excretion becomes 0.92. The difference between the hippuric acid means is not significant.

If the urine volume is very small, less than 50-60 ml., then results should be interpreted with caution and the test repeated with a larger volume. Apart from this, we have found no correlation between volume and test results.

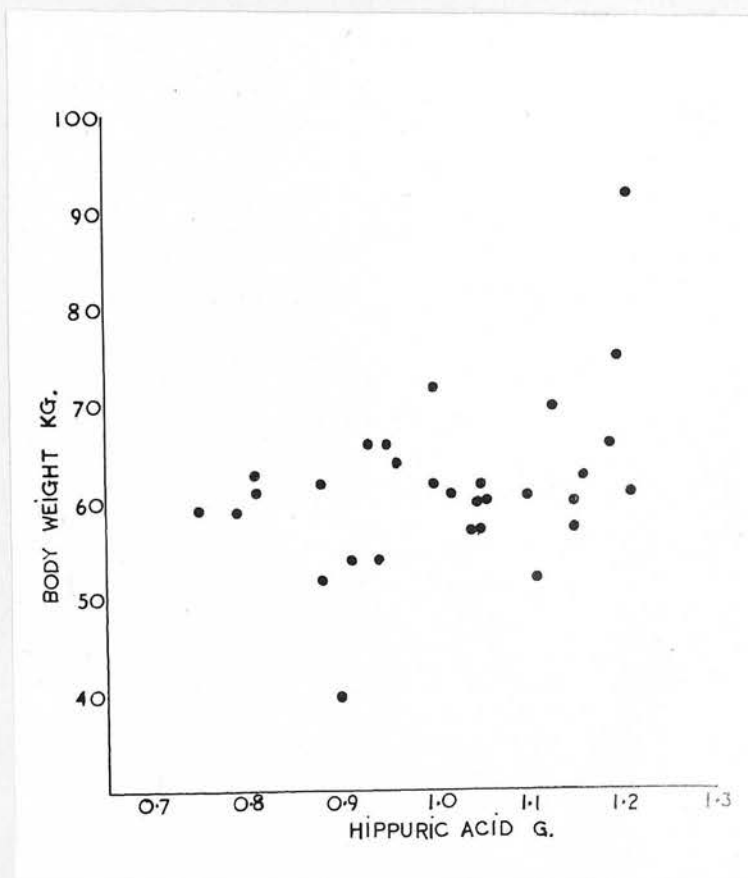
Relation of Body Weight to Test Results.

There is evidence that organ size varies with body weight and surface area (Greenwood and Brown, 1913). The test dose of sodium benzoate is such that a maximum load is put on every liver cell. The larger the liver, therefore, the more hippuric acid can be synthesized. Quick (1931) states that if glycine is given with the sodium benzoate, then the excretion of hippuric acid reaches a maximum varying with the size of the individual and so of his liver. Hepler and Gurley (1942) gave no upper limit for test results because this depends on body size. Machella et al. (1942) could find no correlation between body weight and excretion of hippuric acid. Scurry and Field (1943) studied the matter in more detail. In normal subjects, a significant positive correlation existed between surface area or body weight and the weight of hippuric acid excreted. Formulae were evolved relating these factors.

We have noted the body weight and the hippuric acid excretion in 30 normal subjects (table 18)

Scrutiny of Fig.19 shows that the correlation is very slight ($r = 0.311$, which is not significant).

19.



In subjects of average weight it is unnecessary to allow for varying poundage. In the very large or very small, it is useful to apply the formulae of Scurry and Field when a more accurate estimate of synthesis in that individual is obtained.

The Relation of the Liver to Hippuric Acid Synthesis.

Opponents to the renal theory of hippuric acid formation, especially in man, existed almost from the

outset (Weyl and Anrep, 1880; Kronecker, 1883), and soon physiologists demonstrated the important part played by the liver in the synthesis. Kühne and Hallwachs (1857) found that the synthesis of hippuric acid from benzoic acid occurred in the hepatic vessels in the presence of glycocholic acid. Friedmann and Tachau (1911) isolated hippuric acid after perfusing the liver of a rabbit with blood containing benzoic acid but no glycine. Lewis (1921) inserted a cannula into the common bile duct of the rabbit and demonstrated that synthesis of hippuric acid occurred after exclusion of bile from the intestine.

More indirect evidence came with the discovery that impaired synthesis existed in the presence of experimental liver damage. Lackner et al. (1918) showed that production of hippuric acid varied with the condition of the liver, that of the kidney remaining constant. Dogs poisoned with hydrazine sulphate had diminished excretion despite normal kidneys. Tulane et al. confirmed this work, using the rabbit as the experimental animal. Delprat & Whipple (1921) showed delayed excretion of hippuric acid in liver necrosis produced by chloroform in dogs. Quick and Cooper (1932), however, found that chloroform only slightly decreased the synthesis in the dog and using the same animals Bryan (1925) could find no impairment in synthesis after carbon tetrachloride. Turning to hippuric acid synthesis after the administration of liver poisons to the rabbit more uniform results are reported. Kanzaki (1934) showed that phosphorus and chloroform impaired

hippuric acid production in the rabbit. Adlersberg and Minibeck (1936) reported similar results, using carbon tetrachloride as the toxic agent. Brakefield and Schimdt (1904) injured the rabbit's liver by common bile duct ligation. Montes et al. (1942) showed that the rat behaved rather like the dog in that carbon tetrachloride did not impair hippuric acid synthesis. It seems that there is a species variability in the site of formation of hippuric acid; in dogs the liver is the main site, whereas in rabbits and rats synthesis can occur in the presence of a damaged liver. The validity of these experiments is open to question. The toxic substances may be poisonous not only to the liver but also to other organs, especially the kidneys.

The Mechanism of Synthesis of Hippuric Acid.

The use of hippuric acid synthesis as a test of hepatic function involves the oral or intravenous administration of a definite amount of sodium benzoate followed by the measurement of the hippuric acid excretion in the urine during a given time. Three stages in the process can be separated:

1. Manufacture of Glycine.

(a) Glycine is probably produced in the liver.

Friedmann and Tachau (1911) showed that perfusion of the liver with benzoic acid alone was followed by the appearance of hippuric acid in the perfusing fluid.

The liver had therefore provided the glycine necessary for the synthesis.

Mann and Bollman (1926) report the formation of hippuric acid from sodium benzoate in the hepatectomised dog.

The validity of this work is somewhat nullified by the fact that it was benzoic acid not hippuric acid which was estimated. Free benzoic acid excretion is an indication of liver damage and is probably due to inability of the liver to produce glycine (Bryan, 1925). The work of Mann and Bollman was performed on the dog, an animal in which, as we have seen, hippuric acid synthesis occurs mainly in the kidneys.

- (b) After a dose of sodium benzoate the administration of excess glycine will increase the output of hippuric acid both in experimental animals (Griffith and Lewis, 1923) and in man (Bryan, 1925; Vaccaro, 1935; Yardumian and Rosenthal, 1936; Quick et al., 1938; Probstein and Londe, 1942). Glycine containing proteins act similarly (Lewinski, 1908); Cohn, (1901) showed that feeding proteins would counteract the toxic effect of benzoic acid.
- (c) It appears that, in normal subjects, glycine production is the limiting factor in the rate of synthesis of hippuric acid. Benzoic acid stimulates the liver to manufacture glycine. The body has no great reserves of the substance. Quick (1932) found that provided an adequate dose of sodium benzoate is given, then the excretion of hippuric acid

proceeds at a constant rate irrespective dosage. The normal adult can produce enough glycine to conjugate 0.9 - 1.3 grams of benzoic acid every hour. This figure corresponds well with that of Bignami (1924), namely 21 grams in 24 hours. The mechanism for producing glycine probably has no great margin of reserve (Quick, 1931). This suggests that hippuric acid synthesis might be a sensitive index of liver function.

2. The process of conjugation of the manufactured glycine with the benzoic acid to form hippuric acid.

The site of conjugation probably varies from animal to animal. In the dog and the rat it is mainly in the kidney; in man and in rabbits the liver plays the more important part.

The synthesis resembles the process of conjugation of glycine with cholic acid to make the important bile acid - glycocholic acid. This in man is undoubtedly a liver function. This similarity may mean that estimation of the power of hippuric acid synthesis may indicate the potentialities of the liver for the more essential bile acid production.

Quick (1936) suggests that reduced excretion of hippuric acid in hepatic damage is due not only to impaired glycine production but also to failure of the enzymatic mechanism which unites the benzoic acid with glycine. The small amount of renal enzyme is insufficient for compensation. Probststein and Londe (1942) divided patients with failure to synthesise hippuric acid into two categories. One group would

increase the rate of manufacture if given glycine; in these glycine production was mainly at fault. The other, smaller, group showed no increase with supplements of the amino-acid and in these failure of conjunction of the benzoic acid with the glycine was postulated.

3. The excretion of hippuric acid by the kidneys.

As we have mentioned above, in renal failure with nitrogen retention, hippuric acid is retained in the blood together with urea and other urinary constituents. However a fairly marked degree of renal damage must exist before such retention occurs. Schwei and Quick (1942) have shown that, in man, the power of the kidney to execute Hippuric Acid 2.5 times the power of synthesis.

Excretion of benzoic acid with glycuronic acid.

In certain animals, for example the pig, sheep and dog, benzoic acid is detoxicated not only by glycine but also by glycuronic acid. Results in man are conflicting. Brakefield (1927) and Yardumian and Rosenthal (1936) state that glycine alone is used as a conjugant. Quick (1931) reports that up to 10-12 per cent of dose of benzoate is detoxicated as the glycuronate. Wagreich et al. (1940) give a lower figure; they give 5 per cent as the proportion conjugated as the glycuronate.

It seems, therefore, that conjugation with glycuronic acid is of minor importance in man.

Conclusions from the literature.

1. There is a great species variability in the site of synthesis of hippuric acid. In dogs and rats the kidney is the more important organ, whereas in man, and also probably in the rabbit, the liver is

the chief organ for its formation, the kidney playing a very subsidiary rôle. This may partially explain the apparently contradictory experimental results recorded in the literature.

2. In liver damage there is defective glycine formation and probably also of the enzymatic mechanism uniting the benzoic acid with the glycine.

3. The power of the kidney to excrete hippuric acid is very great. However, in renal disease, especially with nitrogen retention, failure of elimination does occur. This explains the former use of the hippuric acid synthesis test as an indication of renal function.

4. Conjugation of glycuronic acid with ingested benzoic acid occurs in some animals but only to a slight extent in man.

Use of the intravenous hippuric acid test in the diagnosis of liver disease.

TABLE 21

THE RESULTS OF THE INTRAVENOUS HIPPURIC ACID TEST
IN 99 CASES OF LIVER DISEASE.

Diagnosis	Hippuric acid excretion in g.			Range of values in g.					Totals
	Mean	S.E. Mean	Range	0-.39	.4-.59	.6-.79	.8-1.0	Greater than 1.0	
Acute hepatitis	0.63	.055	1.32 - 0.18	6	13	11	7	3	40
Obstructive jaundice	0.47	.083	1.03 - 0.16	10	4	5	2	1	22
Cirrhosis									
Active	0.41	0.08	0.6 - 0.2	4	5	1	0	0	10
Latent	0.87		1.05 - 0.6	0	0	2	4	2	8
Haemolytic jaundice	0.51		1.16 - 0.2	2	1	1	0	1	5
Hydatid cyst	0.96		1.3 - 0.73	0	0	1	1	1	3
Amoebic abscess	0.95			0	0	0	1	0	1
Kala Azar	1.06		1.21 - 0.97	0	0	0	1	2	3
Amyloid disease	0.91			0	0	1	0	1	2
Secondary malignant disease	0.50		1.0 - 0.3	2	2	0	0	1	5

Acute hepatitis and obstructive jaundice. Quick (1933)

reported that the test was valuable in the differentiation of jaundice due to primary liver cell damage from that due to obstruction of the common bile duct. Later workers have been unable to confirm this claim (Snell and Plunkett, 1936; Yardumian and Rosenthal, 1936; Boyce and McPetridge, 1938; Rennie, 1942; Higgins et al., 1944). The results in the present series are shown in Table 21. No distinction between these two types of jaundice can be made on the results of the hippuric acid test.

Cirrhosis of the liver. Quick (1940) found a normal hippuric acid test in the quiescent phases of cirrhosis. Of the 8 cases of latent "compensated" cirrhosis in our series 6 had excretions of hippuric acid greater than the lower normal level. Of the 10 "active" cases all showed diminished excretion (Table 21). It is evident, therefore, that active cirrhosis is unlikely unless the hippuric acid excretion is diminished and that such a diminution does not exclude a diagnosis of acute hepatitis or of obstructive jaundice. Conversely, a normal hippuric acid test will not eliminate a diagnosis of cirrhosis in a compensated or inactive form.

Miscellaneous diseases involving the liver.

Scrutiny of Table 21 shows that liver diseases of various types without jaundice occur with a normal power to detoxicate sodium benzoate. The condition most likely to be associated with a low hippuric acid excretion are malignant metastases in the liver and liver diseases with concomitant anaemia, fever or cachexia. It is difficult to decide whether the low

value is due to the liver condition or is associated with the other features.

Use of the intravenous hippuric acid test in the determination of the extent of liver cell damage.

Acute hepatitis. Wilson (1939) found that the results of the hippuric acid test proved a sensitive reflection of the degree of hepatic damage. Higgins et al. (1944) could find no close correlation between the amount excreted and the severity or duration of the disease, In our series, close agreement could not be established between the severity of the hepatitis and the excretion of hippuric acid (Table 22). In the mildest degree (Grades A) cell damage is minimal, and the main pathological changes in the liver are portal zone cell accumulations. The mean hippuric acid excretion falls within normal limits (0.9 g.). The severer grades (B, C and D) show much lower means. These grades, however, in spite of their increasing extent of liver cell necrosis, do not show decreasing hippuric acid excretions. In fact, there is no significant difference between the mean values in the grades B, C and D. A basic low level (between 0.45 and 0.6g) exists in the severer forms of hepatitis, and excretion does not often fall below this, whatever the severity of the liver damage. It is difficult to account for this finding if hippuric acid synthesis depends only on the liver. In 19 cases in which a further test was available, the hippuric acid excretion had returned to normal before discharge from hospital. In another 5, the test was normal when a follow-up was performed three months later. In 15 cases, tests were performed at weekly intervals during

THE EXCRETION OF HIPPURIC ACID CORRELATED
WITH THE HISTOLOGICAL SEVERITY OF ACUTE HEPATITIS.

Histological grade	Hippuric acid excretion in g.			Range of values in g.					Totals
	Mean	S.E. Mean	Range	0-.39	.4-.59	.6-.79	.8-1.0	Greater than 1.0	
A	0.9	.055	1.3-0.72	0	0	5	5	3	13
B	0.50	.04	0.91-0.18	4	9	4	2	0	19
C	0.52	.053	0.64-0.36	2	3	2	0	0	7
D	0.49			0	1	0	0	0	1

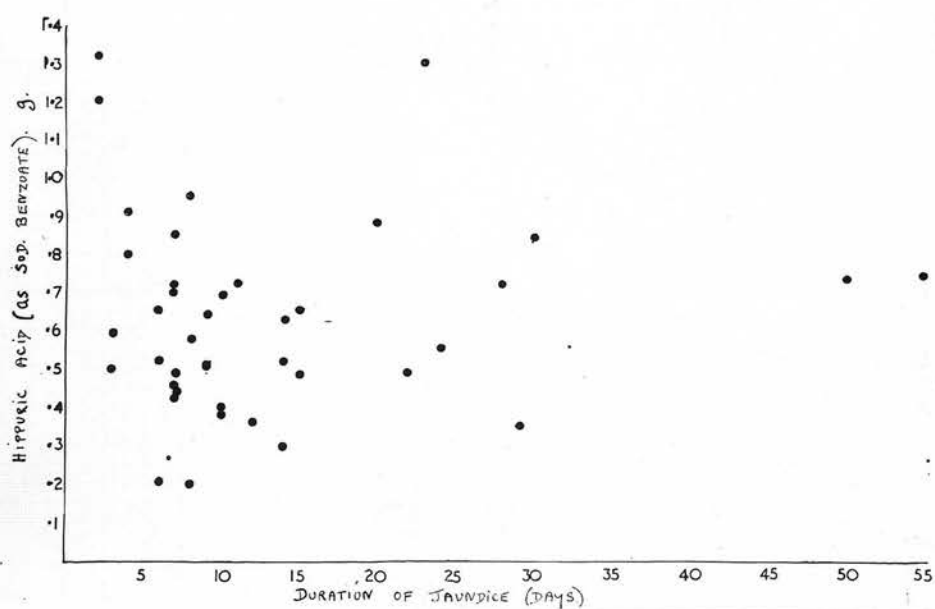
the course of the illness (Table 23 seen on following page). The mean time taken for the hippuric acid excretion to rise from a low to a normal value was 25 days (range 17 - 63). In general, the cases whose livers showed the greatest initial histological severity took the longest time to regain the normal power of synthesis. Riddell and Anderson (1944), in a series of cases of arsenotherapy jaundice, also found a steady return to normal of the hippuric acid test as the patient recovered. Comparison with the recovery rate for galactose (Table 17) shows that the process of hippuric acid synthesis has a slower rate of recovery. Return of normal galactose tolerance did not imply complete normality of liver structure. Return of normal hippuric acid synthesis, however, often followed 2 - 5 weeks after the liver had recovered its normal appearance. This also suggests that hippuric acid synthesis does not depend only on liver changes. In contrast with the results for the galactose tolerance test, there appeared to be no correlation between the excretion of hippuric acid

TABLE 23.

ACUTE HEPATITIS: TIME TAKEN FOR RESTORATION
OF A NORMAL INTRAVENOUS HIPPURIC ACID TEST.

Case	Sex	Age	Histol- ogical Grade	Impaired hippuric acid test			Normal hippuric acid test		
				Duration of jaundice (days)	Serum Bilirubin	Hippuric Acid g.	Duration of jaundice	Serum Bilirubin	Hippuric Acid g.
1	F	20	A	6	3.1	0.65	19	1.5	0.87
2	F	24	A	28	3.2	0.72	44	1.0	0.93
3	M	46	A	7	5.6	0.85	20	1.3	0.95
4	M	41	B	8	8.0	0.21	20	1.2	1.01
5	M	21	B	7	3.5	0.71	23	2.5	0.93
6	M	41	B	14	12.0	0.3	41	2.3	1.1
7	M	18	B	4	3.0	0.8	33	1.3	1.2
8	M	37	B	15	6.4	0.49	34	1.2	0.86
9	M	33	B	10	6.2	0.2	45	2.6	0.90
10	M	27	B	8	8.4	0.18	61	1.4	1.04
11	M	38	C	10	18.0	0.38	17	3.3	0.84
12	M	28	C	10	11.5	0.63	31	1.6	0.84
13	M	41	C	9	12.0	0.40	25	1.5	0.83
14	F	20	C	12	7.1	0.36	50	2.0	1.08
15	M	61	D	7	13.8	0.49	63	0.5	0.86
		Mean		10	8.1	0.49	35	1.7	0.95

ACUTE HEPATITIS: Relation between hippuric acid test and duration of JAUNDICE.



and the period of jaundice at which the test was performed (fig.20).

Cirrhosis of the liver. Biopsy studies using the peritoneoscope indicate that the hippuric acid test results correlate well with the extent of liver cell damage in cirrhosis (Cates, 1941; Gottardo and Winters, 1942). In this series, the cases which were symptom-free, and in which the liver histology revealed no liver cell necrosis, the hippuric acid synthesis was usually normal. The cases with symptoms and in which cell necrosis could be demonstrated in the liver all showed a diminished excretion of hippuric acid. The amount excreted did not correlate with the apparent extent of liver damage or with the eventual prognosis. This again suggests that the hippuric acid synthesis test does not solely measure liver function.

Obstructive jaundice. Study of the literature suggests that the hippuric acid test achieves its greatest usefulness in study of surgical jaundice. Vaccaro (1935) reports that in surgical cases the excretion of hippuric acid compared with gross pathological findings. Snell and Plunkett (1936) found that in surgical jaundice the test is correlated with the degree of hepatic injury, the clinical condition and the post-operative reaction of the patient. Rennie (1942) states that the appearance of the liver at operation is in agreement with test results. Our experience has been that where marked liver changes, such as biliary cirrhosis or metastases, are associated with the obstruction to the common bile duct, the results of the hippuric acid test are usually

low. A low hippuric acid test is, however, not constantly associated with these changes in the liver, and does not necessarily indicate irreversible liver damage if the obstruction can be relieved. The hippuric acid test is so often abnormal in cases of cachexia or of pyrexia that it is difficult to be certain whether the abnormal result is due to these associated features or whether it is actually due to organic liver damage. Boyce and McPetridge (1938) report that in obstructive jaundice the excretion of hippuric acid is proportional to the duration of jaundice, and bears no relationship to the depth of jaundice. In our small series the lower hippuric acid values were found in the cases of the longest duration and with the deepest icterus. This is only to be expected, for these conditions are associated not only with the greatest degree of liver damage, but also with the most general ill-health.

Hippuric acid synthesis in various Non-hepatic Diseases.

Low results for the hippuric acid test have been reported in condition in which the primary emphasis is not usually placed on the liver. Low values have been found in anaemia (Fouts, Helmer and Zerfas, 1937; Henderson and Splatt, 1942). Moser et al. (1942) mention impairment in pregnancy and in diabetes mellitus; Paulson and Wyler (1942) report that patients with gastro-enteric malignant disease not involving the liver gave low results. De Lor and Reinhart (1940) record diminished power of synthesis in general infections and debilitating diseases. Higgins, O'Brien, Stewart and Witts (1944) in their large series encountered reduced

hippuric acid excretion in one case each of myelomatosis, carcinoma and thyrotoxicosis. Campbell (1942) states that low figures follow surgical operations on patients without biliary disease.

Our results have been fully in accord with those in the literature. We have found low results in anaemia of various types, in diabetes, in pneumonia and in malignant disease not involving the liver (Table 24).

TABLE 24

LOW HIPPURIC ACID SYNTHESIS IN VARIOUS NON-HEPATIC DISEASES WITH NORMAL LIVER HISTOLOGY.

Case	Sex	Age	Diagnosis	Hippuric acid excretion g.	Notes
1	M	72	Haemolytic anaemia	0.4	6.24g.Hb. (40%) [*]
2	F	55	" "	0.3	4.01g.Hb. (26%)
3	F	80	Pernicious anaemia	0.2	6.24g.Hb. (40%)
4	M	64	" "	0.6 1.2	7.35g.Hb. (47%) 12.5 g.Hb. (80%)
5	M	17	Iron deficiency anaemia	0.7	7.8g Hb. (50%)
6	M	62	Post-gastrectomy anaemia	0.5	8.0g.Hb. (51%)
7	M	24	Post-haemorrhagic "	0.6 1.2	6.24g.Hb. (40%) 10.1 g.Hb. (65%)
8	F	59	Leucoerythroblastic "	0.7	7.8g. Hb. (50%)
9	M	35	Anaemia, ? cause	0.31	7.8g. Hb. (50%)
10	M	44	Carcinoma of stomach	0.73	Autopsy. No hepatic metastases
11	M	60	Malignant ascites	0.63	" " " "
12	M	50	Carcinoma of lung	0.73	" " " "
13	F	70	Diabetes mellitus	0.33	
14	F	40	" "	0.67	
15	F	42	" "	0.5	
16	M	28	Convalescent pneumonia	0.44	
17	M	50	Afebrile pneumonia	0.62	
18	M	46	Lung abscess	0.60	
19	F	46	Rheumatoid arthritis	0.7	Active phase. Pyrexial.

*

Per cent of Haden normal (100% = 15.6g. Hb.)

CHAPTER X.Biochemical Investigation in Liver Diseases.Discussion and Conclusions.

Clinicians have long been dubious of the practical value of most of the laboratory aids used in the study of liver diseases. This investigation of some of the more commonly used methods, correlated with the histological appearance of the liver, has rather supported this contention. Soffer (1935), in his comprehensive review of liver function tests, states that normal values do not exclude the presence of hepatic disease. This is borne out by our series. All types of liver disease - acute hepatitis, cirrhosis, both primary and that secondary to biliary tract disease, massive malignant metastases and cysts have been associated with normal results for all the techniques studied. Moreover, although the converse is not common, occasional false positives have been observed, especially with the hippuric acid synthesis test. Tables 25 and 26 (see following pages) present a summarised evaluation of the methods of investigation employed.

Serum bilirubin estimations, although of little importance in the differential diagnosis of jaundice, will continue to be used in confirming the clinical impression of icterus, in the estimation of the severity of an acute hepatitis, and in following the course of acute and chronic hepatitis and of obstructive jaundice.

The use of serum phosphatase in the study of jaundice still rests on an empirical basis, but of the methods studied it has proved the most helpful in making a differential diagnosis between obstructive

TABLE 25.

THE DIFFERENTIAL DIAGNOSTIC VALUE OF THE LABORATORY METHODS DESCRIBED.

Diagnosis	Serum Bilirubin mg/100ml.	Serum Phosphatase units/100ml.	Serum Cholesterol mg/100ml.	Serum Proteins		Galactose time (minutes)	Intravenous Hippuric acid test (g)
				Total	g/100 ml. Albumen		
Acute hepatitis	Useless	Usually less than 30.	Usually less than 300	Useless	Useless	Useless	Useless
Cirrhosis Active	Usually less than 12	"	"	"	"	Usually abnormal	Usually abnormal
Latent	Usually less than 1	"	"	"	"	Usually abnormal	Usually abnormal
Obstructive jaundice	Useless	Usually greater than 30	Useless	"	"	Useless	Useless

TABLE 26.

THE VALUE OF THE LABORATORY METHODS DESCRIBED IN ASSESSING
THE EXTENT OF THE SURVIVING LIVER TISSUE.

Diagnosis	Serum Bilirubin mg/100ml.	Serum Phosphatase units/100ml.	Serum Cholesterol mg/100ml.	Serum Proteins g./100ml.		Galactose time (minutes)	Intravenous Hippuric acid test (g)
				Total	Albumen		
Acute hepatitis	High values associated severe lesions	Useless	Useless	Useless	Usually less than 3 in very severe lesions	High values in severe lesions	Useless
Active cirrhosis	Useless	Useless	Useless	Useless	Usually less than 3 in sev- ere cases	Correlates well with severity of lesion	Useless

jaundice and acute or chronic hepatitis. The suggestion of Drill and Ivy (1944), based on animal experiments, that the estimation might be useful in detecting slight degrees of liver damage, has not been substantiated.

Estimation of the total serum cholesterol has proved almost valueless in this investigation. The level is affected by circumstances not directly related to the liver, such as fever, cachexia and starvation. Moreover, the wide normal range of serum cholesterol makes interpretation of small changes difficult.

The serum proteins also reflect general changes as well as liver diseases. However, the normal range in the blood is narrower and the estimation has some place in the study of hepatic dysfunction. A differential estimation is essential. Values of total serum protein give little information either diagnostically or prognostically. The careful differential estimation of serum proteins is a tedious process, and the information given only rarely justifies the time spent in the laboratory. The greatest use arises in assessing the extent and severity of acute and chronic parenchymatous liver disease. A serum albumen of less than 3 g. per 100 ml. is usually associated with an unduly severe lesion.

The results of the intravenous galactose tolerance test do seem to bear a direct relationship to the amount of surviving liver tissue. However, in keeping with the results of other observers, this test has proved insensitive, a severe degree of liver damage being essential for galactose tolerance to

become impaired (Soffer, 1935; De Lor and Reinhart, 1940; Krarup, 1943; Drill and Ivy, 1944). The technique is very time-consuming, both in the wards and in the laboratory, and careful, accurate, technical skill is essential for results to be reliable.

The hippuric acid synthesis test is usually quoted as one of the most sensitive methods of assessing liver function. It is certainly abnormal in a very high proportion of cases of liver disease. This necessarily limits its value in the distinction of one type from another. However, the test is so often abnormal in conditions in which the liver is histologically normal, and in which other tests of liver function (including the bromsulphthalein excretion test) are normal, that it is difficult to incriminate the liver as the only cause of a low test result (Sherlock 1945). This must lessen the value of the method as a measure of the severity of liver involvement in any disease process.

Study of the findings in the series of acute hepatitis cases has failed to reveal any biochemical distinction between "simple" infectious hepatitis and hepatitis following on arsenotherapy or the parenteral administration of known icterogenic sera. The arsenotherapy cases, however, have usually fallen into the severer histological grades.

There have been a great many papers comparing the sensitivity of the various methods used to estimate liver function. Mann (1941) stresses the dissociation of liver functions so that a test of one property, for instance the power to store glycogen in hepatic cells, may be abnormal, whereas another testing, for

example detoxicating power, is normal. Drill and Ivy (1944), on the other hand, stress the association of liver functions and liver function tests, more of which become abnormal as the degree of liver damage increases. Results obtained in this series rather favour the latter view. Low results for the hippuric acid synthesis test are an early abnormality in liver disease, followed by changes in galactose tolerance, and only in the very severe cases does the serum albumen level show a marked fall. This suggestion would need substantiation by the use of additional techniques and by a larger series. The hypothesis, moreover, cannot be verified by reference to the histological appearances of the liver, for the results of the tests cannot be identified exactly with changes in liver histology. Certainly, the more laboratory investigations pursued, the greater likelihood is there of a biochemical deficiency being demonstrated. To the clinician, anxious to make a diagnosis and to assess prognosis, this type of "shot-gun" investigation may well add to the confusion rather than give a clear-cut answer. The "association" and "dissociation" theories of liver function are based on the results of animal experiment. In man, conditions are much more complex. Dehydration, cachexia, fever and renal disease are frequent accompaniments of human hepatic disease. A pure hepatic lesion is rare. Of the estimations studied, only the serum phosphatase and the intravenous galactose tolerance test seem free from changes brought about by these non-hepatic factors. Before final judgement can be passed on the usefulness of these laboratory aids described above, much more

information is needed of their exact relation to hepatic damage in man. In the meantime, in the jaundiced subject, a single venous blood sample which is analysed for serum bilirubin and serum alkaline phosphatase gives as much information to the general clinician as the more complicated procedures. If facilities are available, the differential serum proteins may be estimated and may give some indication about the severity and prognosis of acute and chronic parenchymatous liver disease. Very similar conclusions were reached by Higgins and his associates (1945). A more satisfactory picture is given if these investigations are repeated at weekly intervals during the course of the illness. In the non-jaundiced subject the biochemical methods enumerated have proved of little value.

ACUTE HEPATITIS

Epidemics of jaundice have always occurred in armies and particularly during times of war. A very large epidemic occurred in the American Civil War (Woodward 1863). Four thousand cases were reported in the Franco-Prussian War (Seggel 1872, Hübener 1917). The South African War had its epidemic (Willcox 1916), and in the 1914-18 conflict outbreaks were encountered on both sides (Brugsch & Schürer 1919, Macpherson 1922). It is therefore not surprising that jaundice proved one of the most important diseases in the war just ended (Witts 1944). Forces in the middle East (Cameron 1943) and in particular the New Zealand and Australian divisions were so affected that at one time more troops were going sick with jaundice than could be replaced. Italy was another theatre of hostilities where hepatitis was particularly prevalent and here the Canadian Forces provided the most victims, the exact number is still a military secret but it is believed to be over 100,000. The allied troops were not the only sufferers and the German Armies also reported epidemics (Dietrich, 1942). Added to these "spontaneous" epidemics were the groups following the parenteral administration of icterogenic sera. The epidemic following the use of yellow fever vaccine affected mainly United States personnel (Fox, Manso, Penn and Pará 1942; Turner et al 1944; Hayman & Read, 1945). Another epidemic was associated with mumps serum inoculations (Hawley et al, 1944), and another followed serum and plasma transfusions (Beeson, 1943, Ministry of Health memorandum, 1943; Morgan & Williamson 1943). At the same time jaundice associated with

arsenotherapy was spreading through our venereal disease clinics. Marshall (1943) gives an incidence rate of 29 per cent in 940 syphilitic soldiers attending 3 clinics. Dudley (1943) states that 30-40 per cent of seamen attending Naval V.D. clinics contracted jaundice. In this war jaundice has ranked second only to malaria as the medical condition with the highest incidence in the Forces. It seemed important therefore to determine the pathological picture of the various types of jaundice and to decide their relationship to the epidemic jaundice related to bile duct blockage ("catarrhal jaundice") first described by Virchow in 1865.

The condition is so rarely fatal that a series of satisfactory dimensions is collected with difficulty from the postmortem room. Cases submitted to exploratory laparotomy are few and usually clinically atypical. The pathology of the condition had already been studied using the aspiration biopsy technique' (Roholm & Iversen, 1939). The present work simplifies and extends these findings and establishes a few clinical conditions. The first part will deal with the general pathological picture of the liver and the second with the general course of the disease and its possible sequelae.

PART I

General Pathology of Acute Hepatitis

Epidemic, arsenotherapy and serum jaundice. Adult cases of epidemic hepatitis admitted to this hospital during the years 1942-45 have been studied. The Army authorities co-operated in the investigation by putting at our disposal cases of jaundice developing

during arsenotherapy for syphilis. The American Red Cross Harvard Unit also invited us to apply the method in case of hepatitis following the inoculation of mumps convalescent serum. To this group we add 5 cases of jaundice which followed serum transfusions in this hospital. The cases can be tabulated as follows:

	<u>Cases</u>	<u>Biopsies</u>	
Epidemic hepatitis	23	29	111
Arsenotherapy jaundice	54	71	
Serum jaundice	10	11	

Although there may be different aetiological factors in each of the above groups, we have not found any recognisable histological criteria for their differentiation. A hepatic inflammation of varying intensity and distribution is common to them all, and therefore for purposes of pathological description we may disregard this aetiological grouping and consider the findings as a whole.

PATHOLOGY

The changes in the liver are related to the severity and duration of the disease. For purposes of comparison, we have dated the condition from the onset of jaundice, although there is always a variable period of diverse prodromal symptoms.

Broadly speaking, the picture is one of hepatic cell necrosis and autolysis, associated with leucocytic and histiocytic reaction and infiltration. The centres of the lobules show the first of these changes most markedly, and the portal tracts the greatest cellular infiltration. In certain cases, which seem to be either those which are mild from the beginning

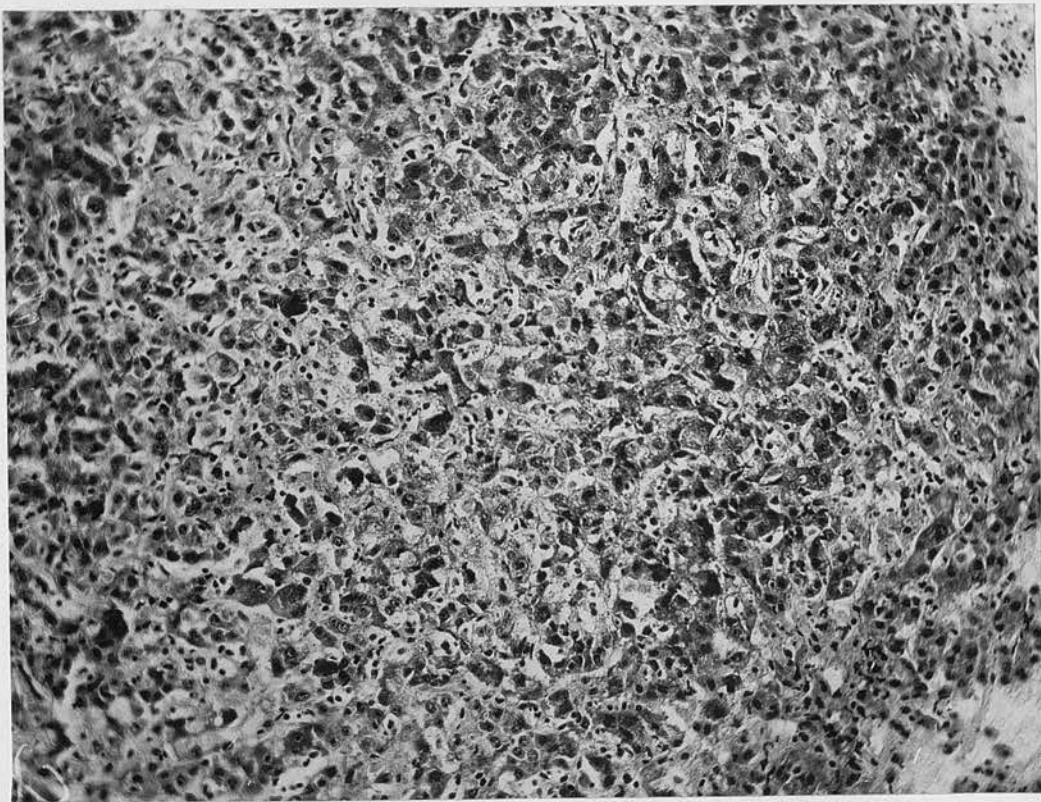
or in which the lesion is retrogressing, the periportal cell accumulations predominate in contradistinction to the more severe cases in which hepatic cell degeneration is more pronounced and the histiocytic and leucocytic infiltration more widespread (figs. 35 & 32). For descriptive purposes, we call the first of these the "zonal" and the second the "diffuse" type of change, but it must be understood that there are no good reasons for regarding these differences as fundamental, and that an intermediate picture occurs which we have denominated a "mixed" lesion. Finally, there are those cases in which the lesion is well on in the phase of retrogression, or is progressing to a stage of necrosis, nodular hyperplasia or cirrhosis.

Frequency with which the different pathological lesions were found in relation to duration of disease and intensity of the jaundice.

Type of lesion	Duration in weeks			Serum bilirubin (mg. per 100 ml)
	Under 1	1 - 2	Over 2	
Diffuse	14	6	1	3-18 (mean 10.0)
Mixed diffuse and zonal	9	7	4	2.6-14.6 (mean 8.7)
Zonal	16	4	13	Under 1 wk. 2.5-6.3 (mean 4.1) Over 2 wks. 1.1-9.2 (mean 3.7)
Residual fibrosis	-	-	13*	

* Duration 4-26 weeks; only 4 slightly jaundiced.

In the table it will be noted that diffuse lesions are seldom encountered after the second week, and that zonal inflammation tends to fall into two groups,



21.

Case 1.

Epidemic hepatitis. Diffuse acute hepatitis showing loss of lobular pattern, irregularity in cell staining with loss of glycogen, and the presence of inflammatory cells.

Stained Best's Carmine. X 160.

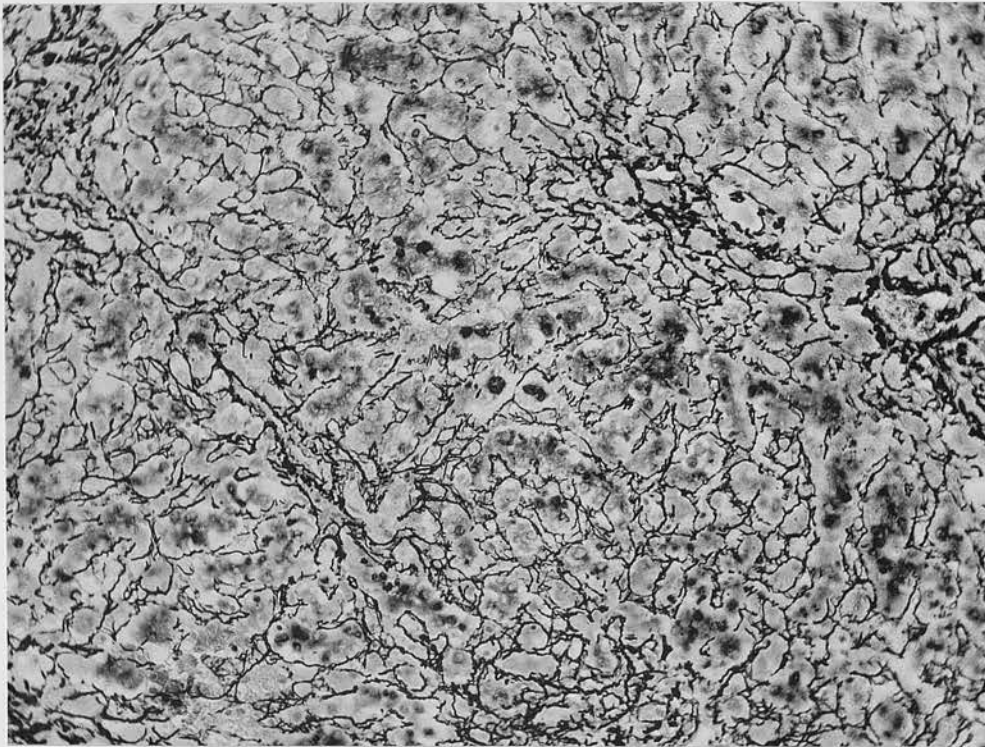
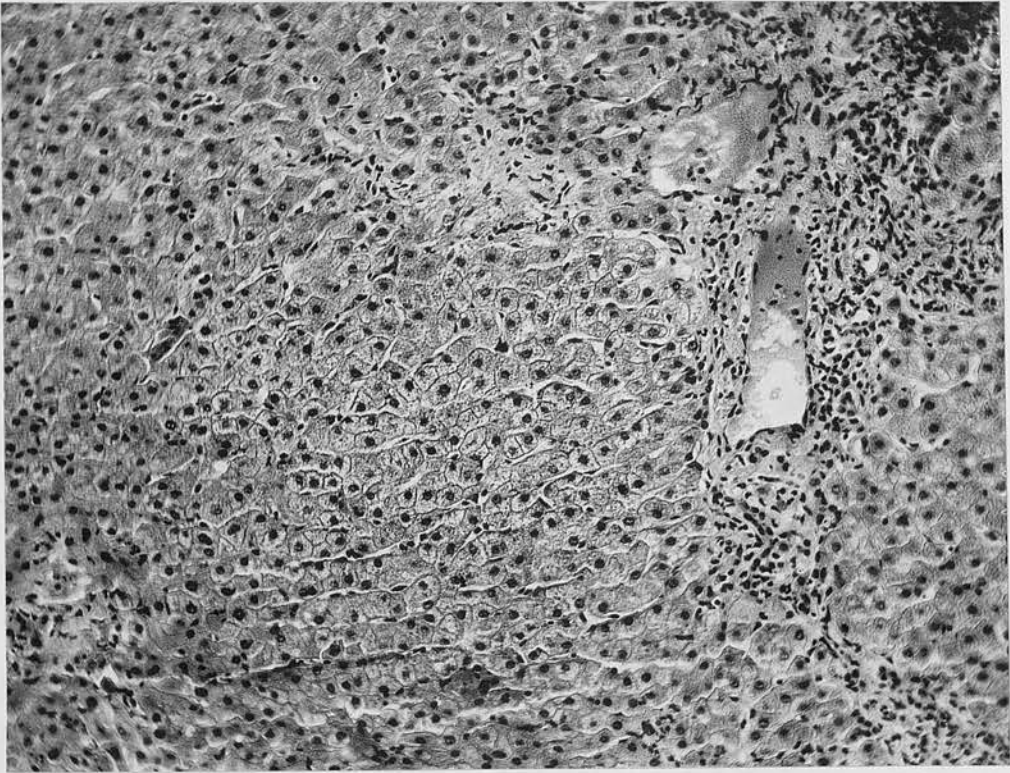


FIGURE 22.

Case 1.

Foot's reticulin stain showing general preservation
of reticulin pattern in spite of the severe cellular
lesion.

X 160.



23.

Case 1.

One month later. Recovery with restitution of the lobular pattern and disappearance of the inflammatory cells. Some increased cellularity remains in the portal tracts.

H.E. X 160.

one of short and the other of long duration. These correspond respectively to early and mild lesions, and late residual lesions in cases which at an earlier stage presumably showed more extensive hepatic change.

Histological Examples

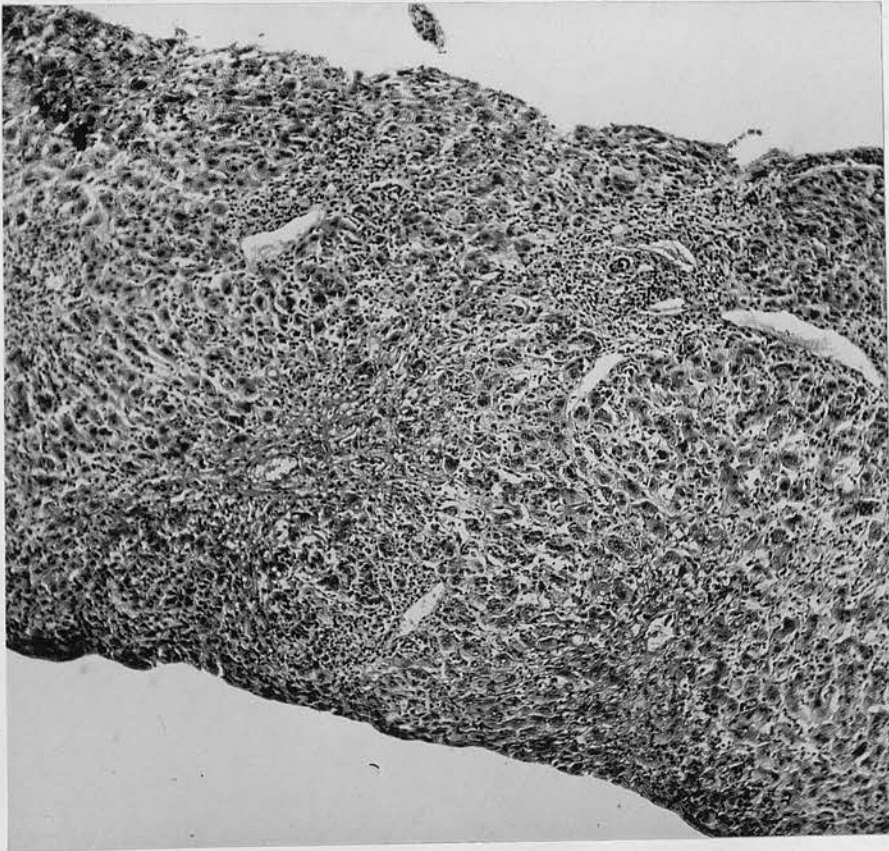
We may illustrate the different degrees of intensity of the liver damage by reference to typical cases.

1. Severe, acute hepatitis, with a diffuse lesion affecting the whole liver lobule.

Case 1. A man, aged 29, suffering from epidemic hepatitis. Jaundiced 19 days. Liver enlarged 3 cm. below umbilicus, serum bilirubin 11.9 mg. per 100 ml. Nausea and vomiting persisted one week after biopsy (figs 21, 22). The section at the acute stage shows an acute diffuse inflammation (fig. 21). (1) The lobular pattern is barely recognisable; the liver cell columns are broken up; the degenerated liver cells are replaced by a tissue made up of collapsed sinusoids, with their reticular framework, and endothelial cells. (2) Many of the surviving liver cells show various changes of necrosis and autolysis, with pyknotic nuclei and evidence of cell proliferation in the shape of numerous binucleate liver cells.

This type of picture has been encountered in 21 cases; it is one from which complete recovery may, and in most cases does, occur. The process of recovery is discussed in a later section.

An even more severe diffuse lesion is illustrated in fig. 24 Case 2. A male, aged 22, suffering from arsenotherapy jaundice. Jaundiced 5 days, liver enlarged to umbilicus, serum bilirubin 14.2 mg. per



24.

Case 2.

Arsenotherapy jaundice. Diffuse form of acute hepatitis with destruction of liver architecture, extreme cell necrosis and infiltration with inflammatory cells.

H.E. X 75.

100 ml. In addition to the diffuse inflammation (similar to that of fig.21) the portal tracts show much infiltration with leucocytes and histiocytes.

This unusually severe lesion did not recover in the usual manner. The jaundice ran a long course. Free fluid was detected in the abdomen 3 weeks later. Finally both the jaundice and ascites cleared, and from the clinical point of view recovery seemed ultimately satisfactory. It is quite possible that nodular hyperplasia and a true cirrhosis may have developed in this patient (see later).

Case 3. A 59 year old man was receiving arsenic injections for syphilis. Jaundiced 11 days. Temperature 101°. Liver enlarged to the umbilicus. Spleen just palpable. Serum bilirubin 15mg/100ml.

Liver Sections (fig.25) show the hepatic damage to be so severe as to be hardly compatible with life. The lobular architecture is lost. Only an occasional group of liver cells survive and many of these are undergoing necrosis. The intervening areas are occupied by inflammatory cells of all types, occasional ghost-like remains of necrotic liver cells can be recognised.

This very severe lesion, after a rather prolonged course (see on) eventually recovered.

The final case in this "diffuse" group is of a severe hepatitis ending fatally.

Case 4. A 27 year old soldier suffered from arsenotherapy jaundice. Jaundiced 3 days. Liver enlarged 4 cm. below the costal margin. Serum bilirubin 9.4 mg. per 100 ml.

FIG. 25.

Case 3. Biopsy 1.

Very severe diffuse hepatitis. Only an occasional isolated group of liver cells survive.

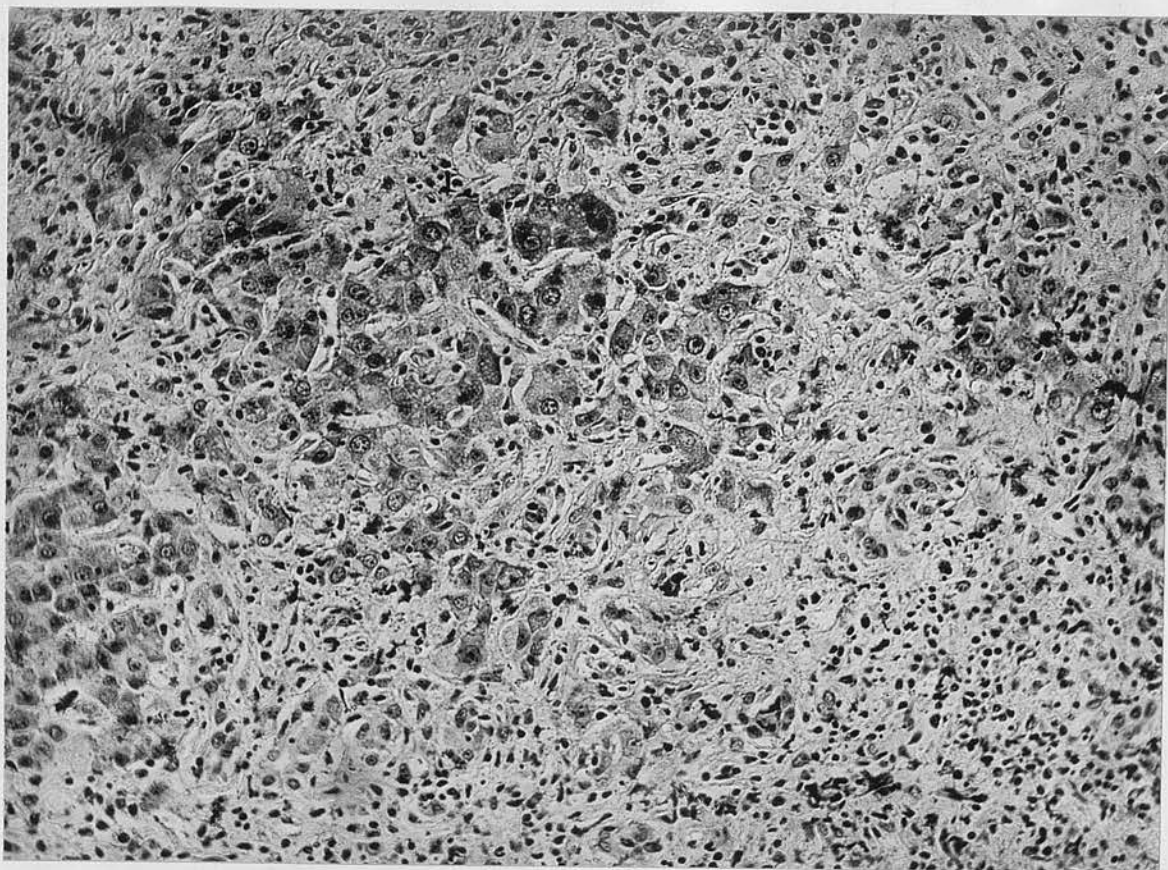
stained Best's Carmine. X 220.

FIG. 26.

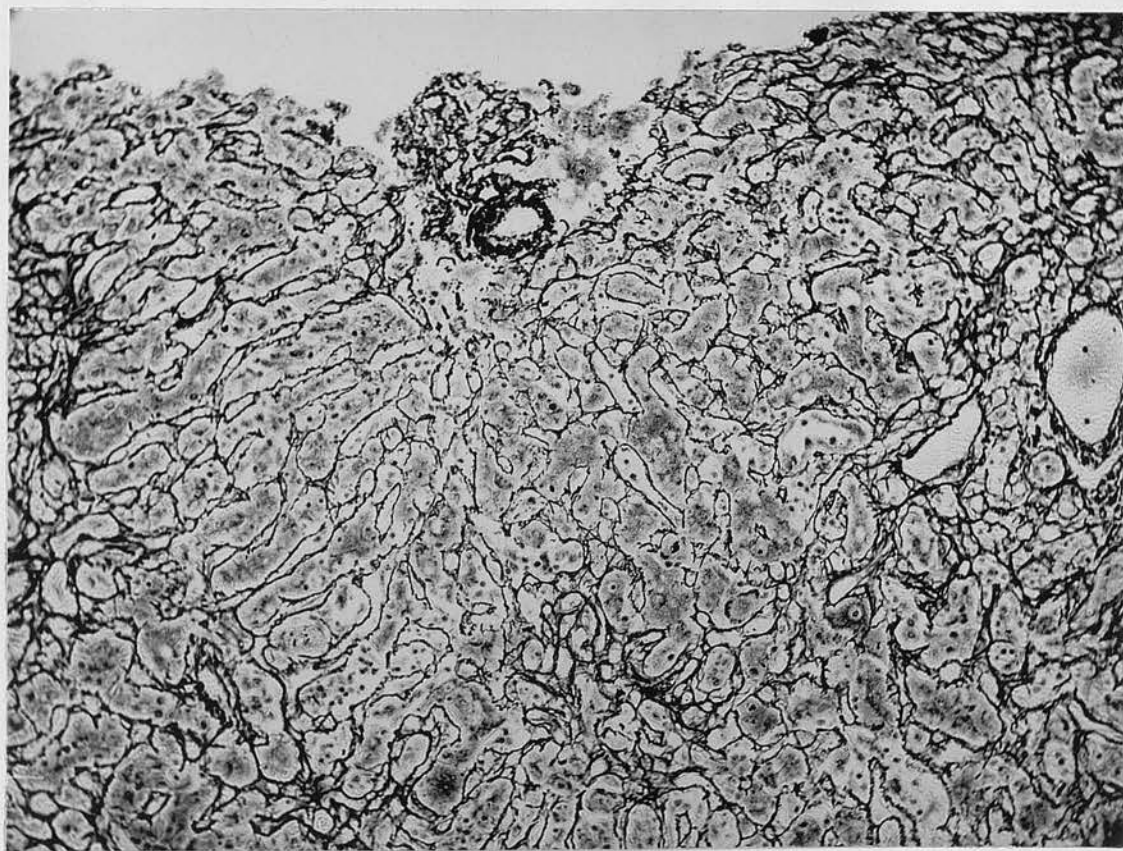
Case 3. Biopsy 1.

Reticulin framework well preserved. Some condensation of reticulin in the central areas and around the portal tracts.

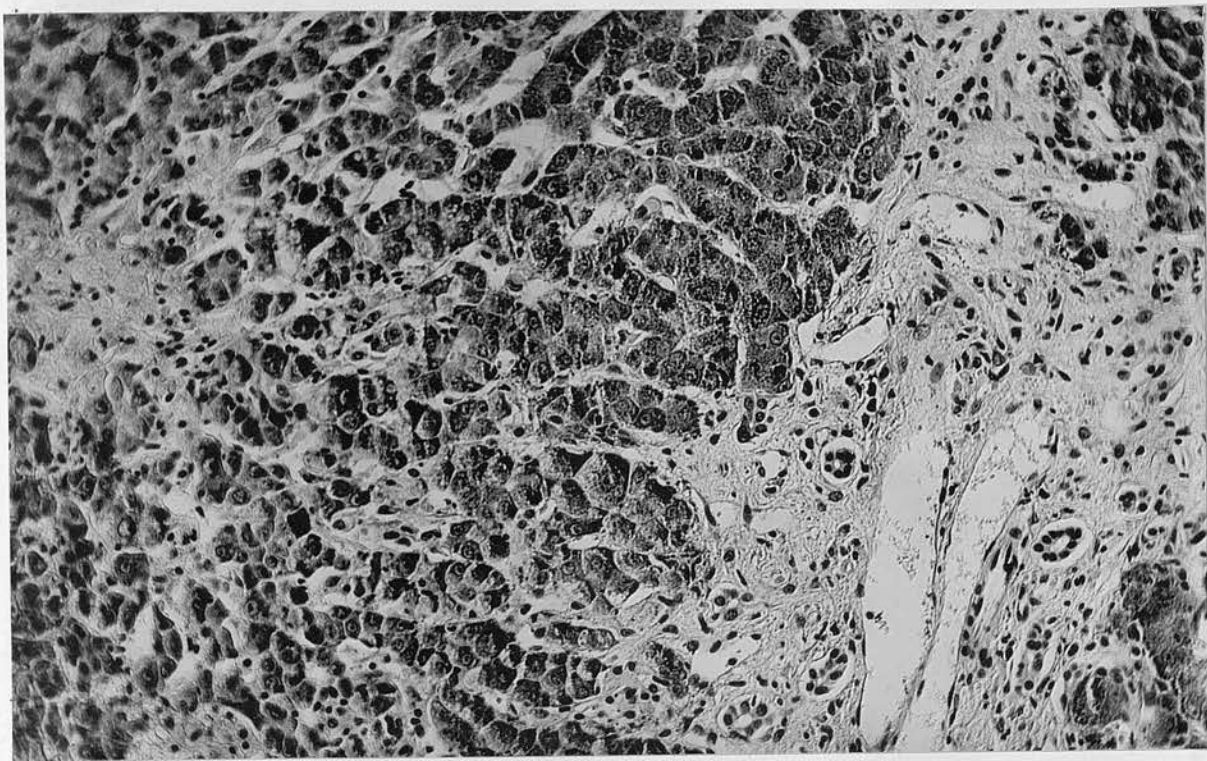
Modified Foot's reticulin stain. X 160.



25.



26.



27.

Case 3. Biopsy 2.

Remarkable degree of recovery. Centre of lobule still shows cell necrosis and inflammatory change.

Portal tracts show reticulin condensation and contain proliferating bile ducts.

Best's carmine stain. X 220.

FIG. 28.

Case 3. Biopsy 3.

Further recovery. Central areas now intact.

Portal tract infiltrations still seen.

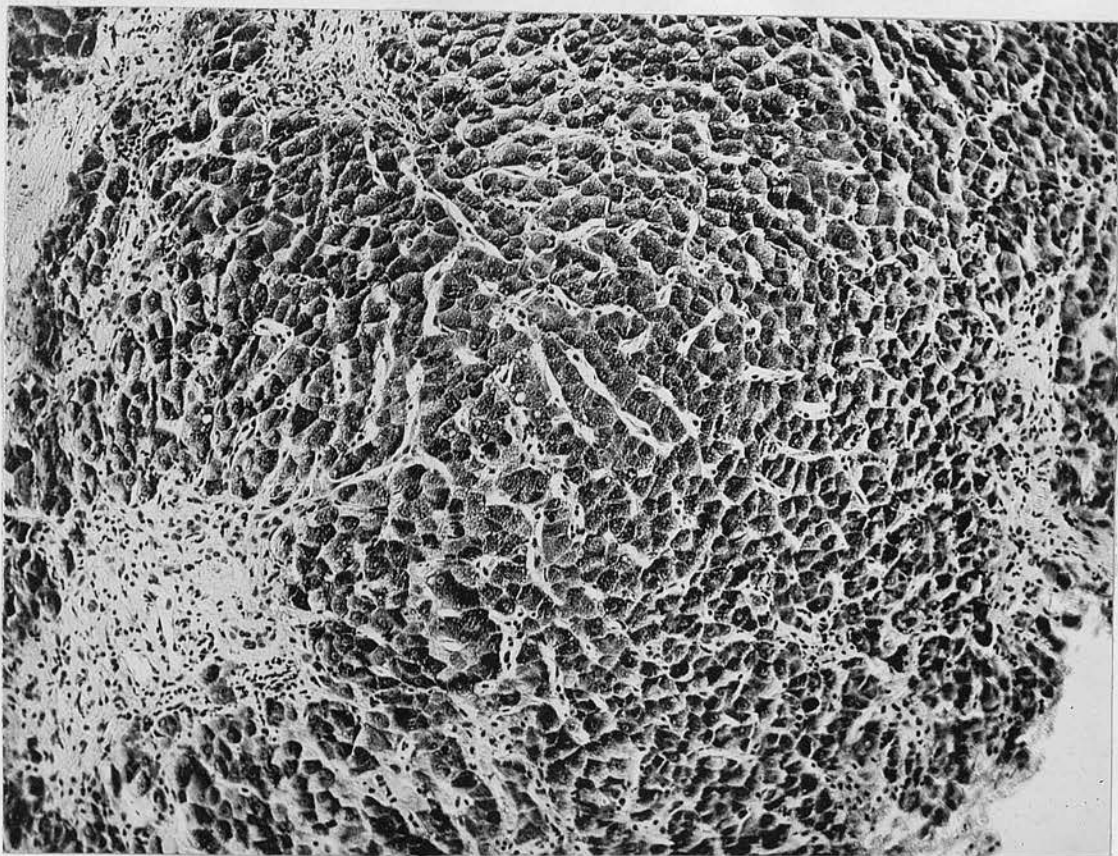
Best's carmine stain. X 135

FIG 29.

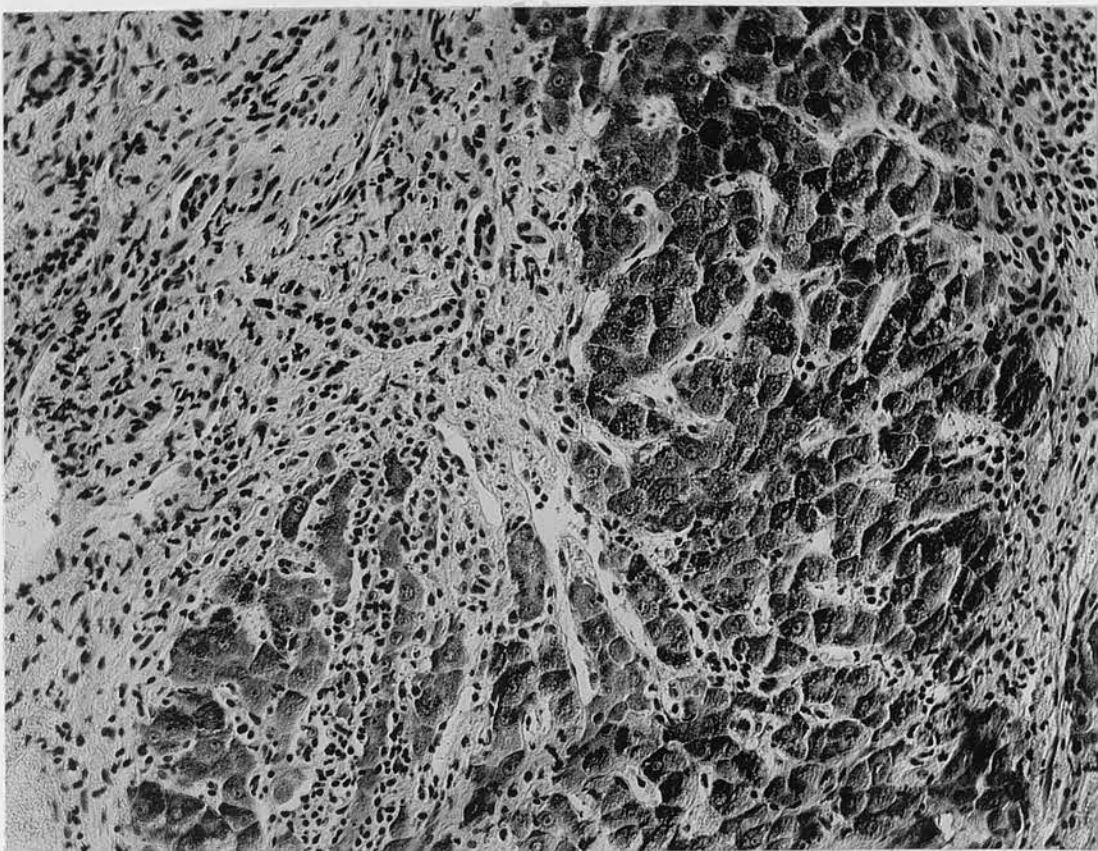
Case 3. Biopsy 3.

Portal tract showing proliferating bile ducts and strands of fibrous tissue passing centrally and to adjoining portal tracts.

Best's carmine stain. X 220.



28.



29.

FIG. 30.

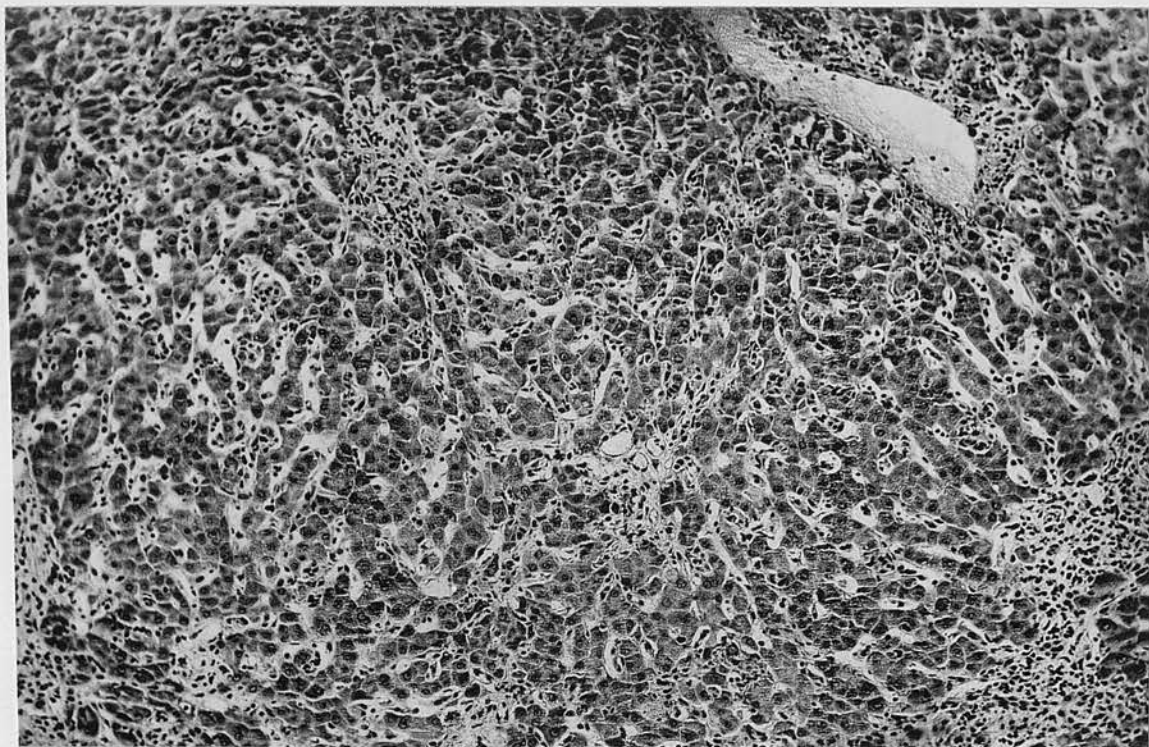
Case 3. Biopsy 4.

Restoration of liver architecture. Histiocytic proliferation in sinusoids and portal tracts. Slight portal tract fibrosis. Best's carmine stain. X 120.

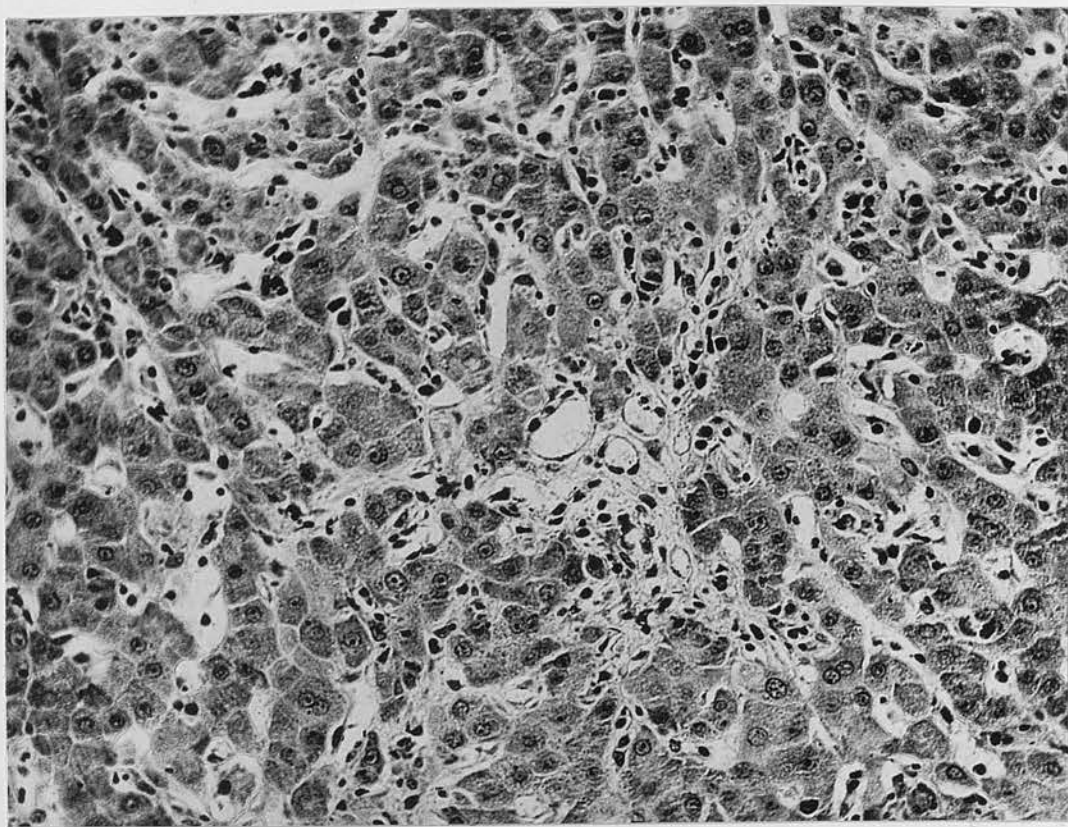
FIG 31.

Case 3. Biopsy 4.

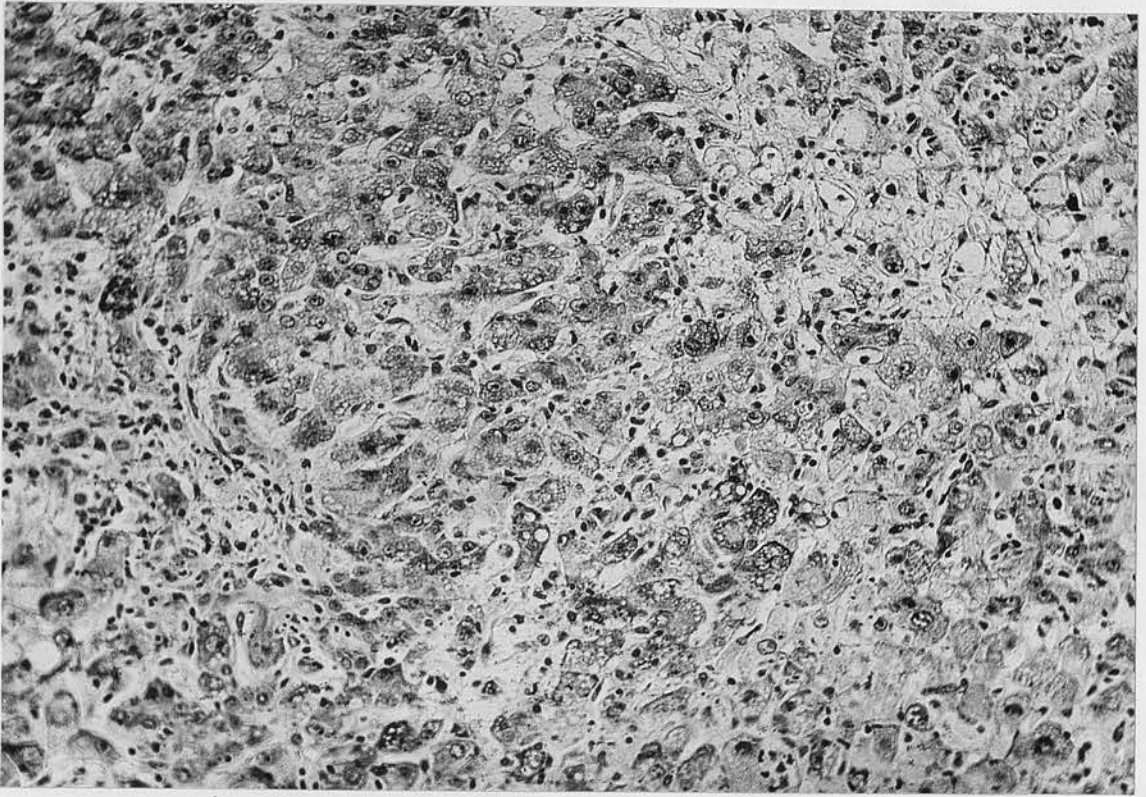
Central area shows excess fibrous tissue. Histiocytic proliferation in the sinusoids. Best's carmine stain. X 250.



30.



31.

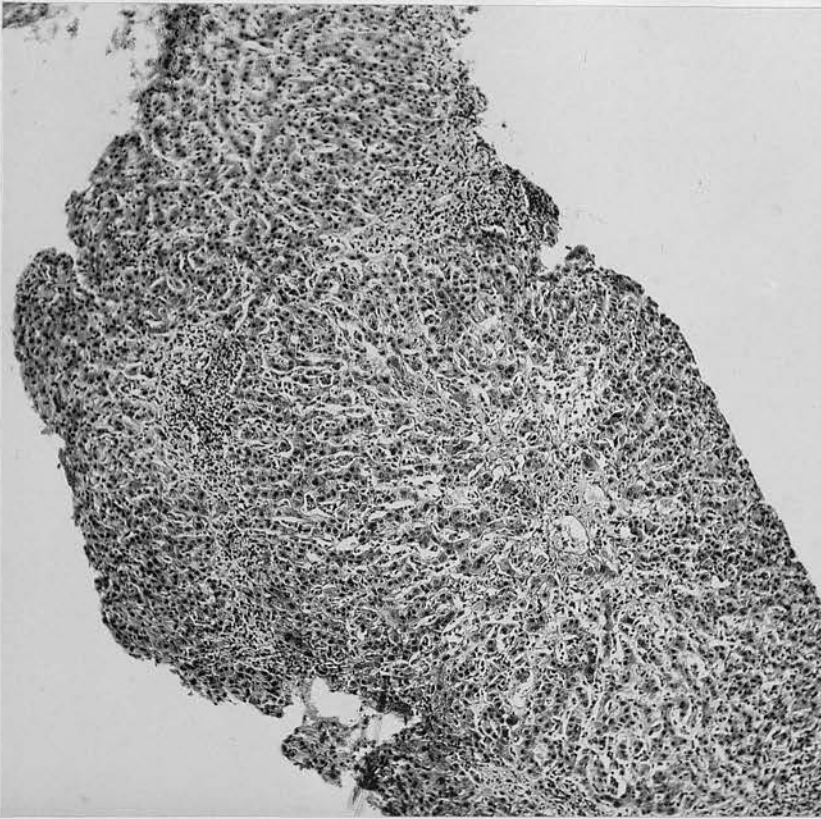


32.

Case 4.

Arsenotherapy jaundice. Diffuse lesion showing loss of lobular pattern. Many cells have lost their glycogen, some contain fat.

Stained Best's Carmine. X 190.

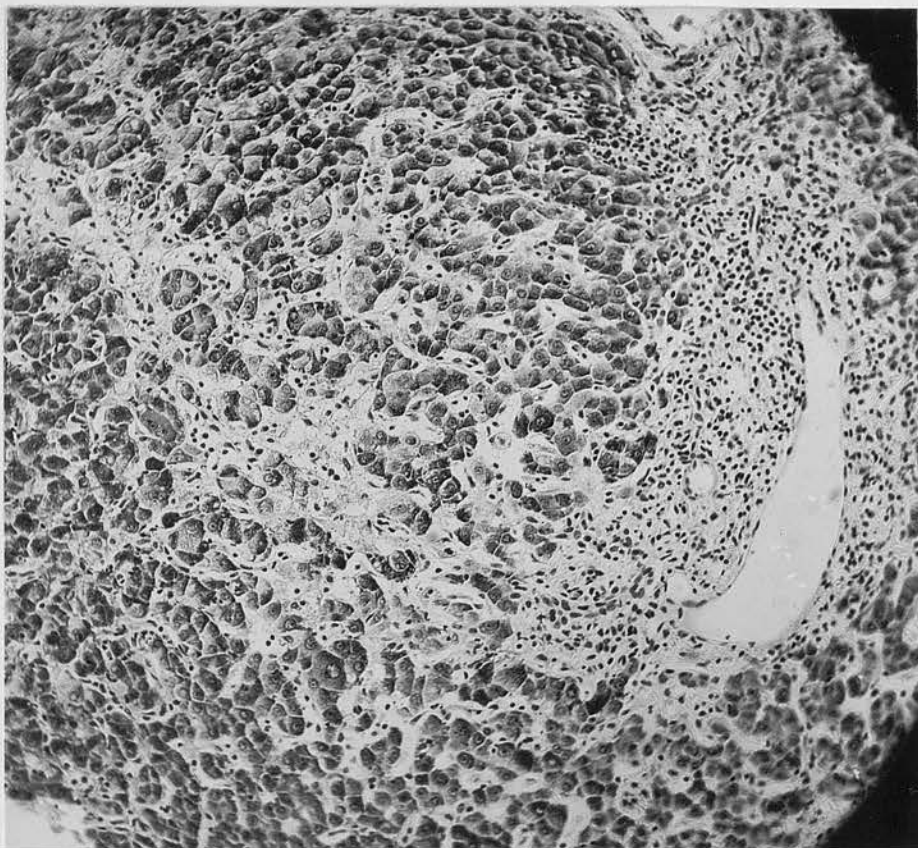


33.

Case 5.

Mumps serum jaundice. Combined focal and diffuse lesion. A central vein is seen surrounded by an area of cell autolysis. A portal tract above shows leucocytic infiltration. The liver cells columns are partially disrupted and there is extensive sinusoidal cell proliferation and leucocytic infiltration.

H.E. X 150.



34.

Case 6.

Arsenotherapy jaundice. Mixed zonal
and diffuse hepatitis.

Best's carmine stain. X 145.

The liver section (fig.32) presents a picture very similar to that shown in fig.25. In addition fatty change is noted in some of the liver cells and some of the cells also show glycolysis. The reticulin framework is reasonably well preserved.

The patient went into coma and, therapy proving unavailing, died 3 days later.

It is interesting to note that in cases 3 & 4 although the sections of liver presented similar histological pictures, the lesion proceeded on such different courses, the one to total recovery the other to death. Treatment was similar. In those cases of maximum histological severity the outlook is obviously worse than for those with minimal hepatic lesions; the fate however cannot be predicted only on the appearance of the liver sections.

2. Moderate acute hepatitis with mixed diffuse and zonal lesions. This picture was found in 20 cases. Here the destruction of the liver lobule is less well marked, and the portal infiltrations are more conspicuous. The histological severity of these cases in general falls into an intermediate class between those presenting the very severe lesions just described and the third group, in which the most obvious changes are in the portal zones.

Case 5. - A man aged 19. Jaundice had followed 10 weeks after the injection of convalescent mumps serum. At the time of biopsy the patient had been icteric for 3 weeks. The serum bilirubin was 14.6 mg. per 100 ml. The liver section is shown in fig. 33 which includes a central vein and a portal tract. The points

which appear are: (1) considerable loss of regularity of liver cell columns and disturbance of the normal architecture of the hepatic lobule; (2) a rarefaction of the cells about the centre of the lobule and some loss of staining - the effects of cellular necrosis and autolysis; (3) an infiltration with small inflammatory cells in the portal zone; and (4) increased cellularity over the whole field due to leucocytic infiltration and some proliferation of the reticulo-endothelial cells of the liver sinusoids. In this picture both diffuse and zonal changes are combined. The lesion is of moderate severity, but the patient made an uninterrupted and clinically complete recovery.

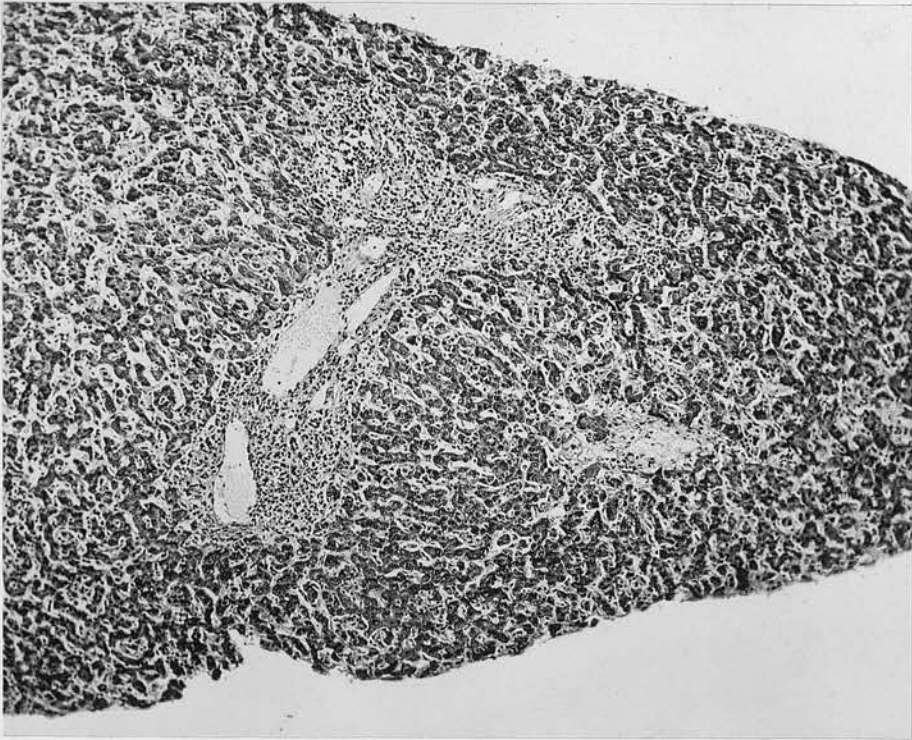
Case 6. Soldier aged 37 with arsenotherapy jaundice. 10 days jaundiced. Liver enlarged 5 cm. below the costal margin and tender. Serum bilirubin 6.4mg/100 ml. The liver section is shown in fig. 34. The points described in fig. 33 can be distinguished in this case. Portal zone cellular infiltrations are perhaps more conspicuous. The glycogen content of the liver cells is well maintained. Suitable stains showed little disturbance of the reticulin pattern.

The patient made an uninterrupted recovery

3. "Zonal" limited type of hepatic lesion.- As we have explained, a lesion predominantly in the portal zones occurs in two circumstances. First, it may be evidence of a mild hepatitis in which the lobular pattern is not disturbed and liver cell necrosis is at a minimum. Secondly, it represents a late condition in a hepatitis which is recovering, and it may be associated with other chronic changes.

The early zonal lesion may be illustrated by the following cases:

Case 7.- A man, aged 32 suffering from arsenotherapy jaundice. Jaundiced 3 days, serum bilirubin 6.1 mg. per 100 ml. The liver (fig. 35) at this magnification shows, as the outstanding change, a cellular infiltration in the portal tracts. The lobule pattern is well preserved, and the cells, stained darkly by Best's method, show their normal load of glycogen. Examination at higher powers almost invariably reveals

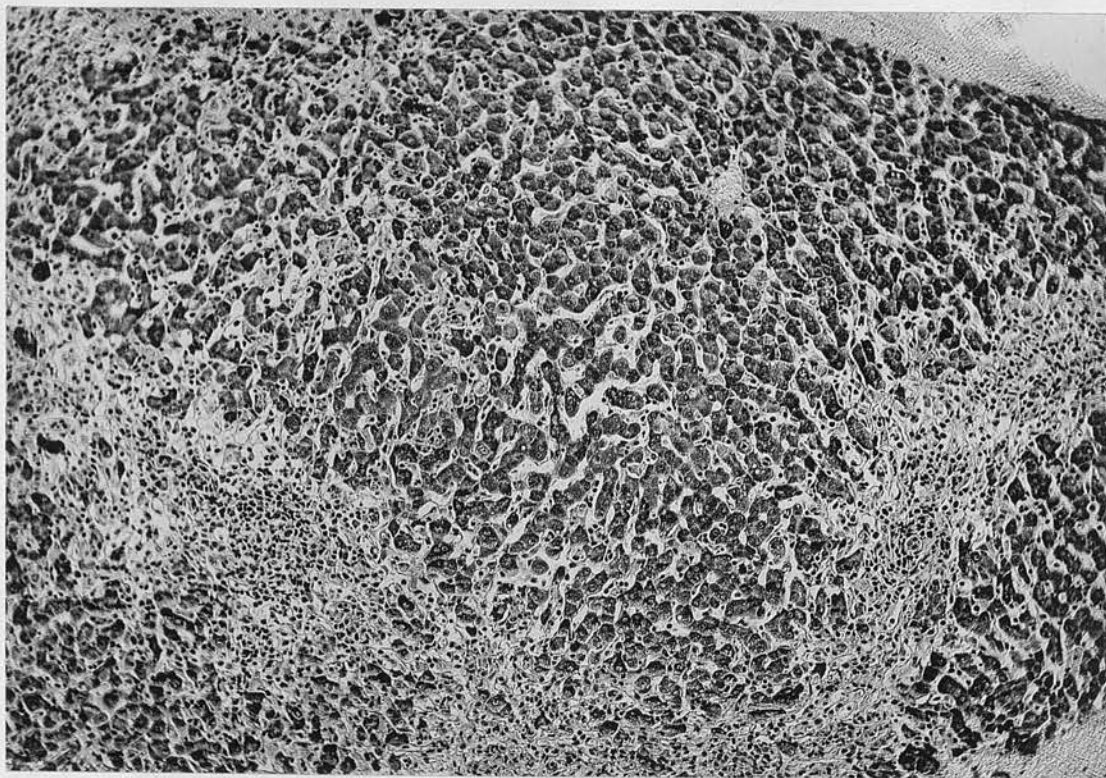


35.

Case 7.

Arsenotherapy jaundice. There is marked cellular infiltration in the portal tracts, the lobule pattern is well preserved and the cells stained darkly by Best's method show their normal load of glycogen.

x 80.

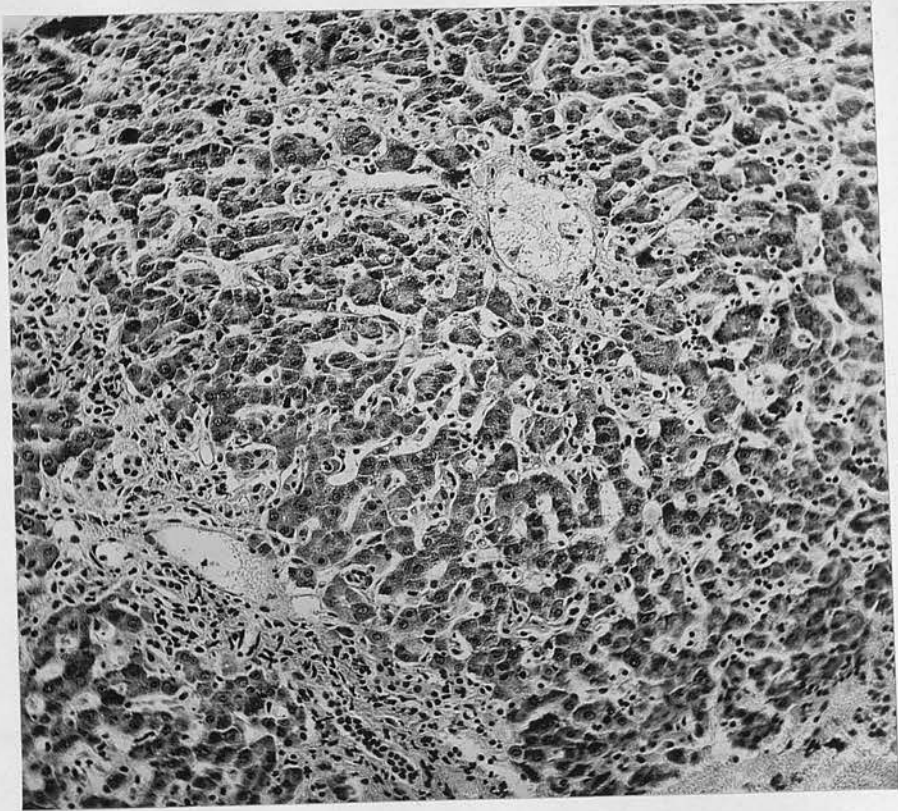


36.

Case 8.

Epidemic hepatitis. Zonal type of hepatitis. Well marked portal tract cellular infiltrations. The adjacent portal tracts have been zoned by areas of cellular infiltration and autolysis. Lobular pattern well preserved.

Stained Best's Carmine. X 105.



37.

Case 9.

Epidemic hepatitis. Lesion affecting mainly the centre of the lobule. Central vein shows hyaline thickening of the wall.

stained Best's Carmine. X 145.

some of the features we have described in the more severe cases, such as a certain amount of cell autolysis around the central vein, some proliferation of the Küpffer cells of the sinusoids, and some evidence of liver cell multiplication; but the outstanding feature is the cellular periportal accumulation of histiocytes, polymorphonuclear leucocytes and a certain number of eosinophils, which gives this picture a peculiarity of its own.

Case 8.- A girl aged 20 suffering from epidemic hepatitis. Jaundiced 6 days. Liver palpable 2 cm. below the right costal margin. Spleen easily palpable 2 cm. below the left costal margin. Serum bilirubin 3.1 mg. per 100 ml.

The liver biopsy section (fig. 36) shows a sharply zonal portal lesion. There is a linking up of the adjacent portal zones by areas of cellular infiltration and autolysis. The trabecular structure of the lobule and the centres of the lobule appear normal. Even at this magnification an abnormal number of liver cells in mitosis can be seen.

This patient made an excellent clinical recovery and in 3 weeks the serum bilirubin was 0.9 mg. per 100 ml. However 2 months after discharge she returned with a further typical attack of infective hepatitis from which she recovered in 3 weeks. The cause of the relapse in infective hepatitis is not known. It does not seem to be related to the severity of the initial attack. A second biopsy was not possible in this patient. However in other cases the picture has not differentiated from an acute hepatitis originating de novo.

Case 9.- A man aged 46 suffering from infective hepatitis. 7 days jaundiced. Liver enlarged 2 cm. below the costal margin. Serum bilirubin 5.6 mg. per 100 ml.

Liver section (fig. 37) shows a zonal hepatitis but unlike the previous examples the zone chiefly affected is the centres of the lobule. In this area many liver cells have disappeared. Other cells are in the process of necrosis and contain quantities of pigment. Portal tract cellular infiltrations are minimal.

FIG. 38.

Case 10. Biopsy 1.

Epidemic hepatitis. There is a wide zone of periportal cellular connective tissue with numerous bile ducts. The lobule shows diffuse inflammatory change becoming more intense towards the centre.

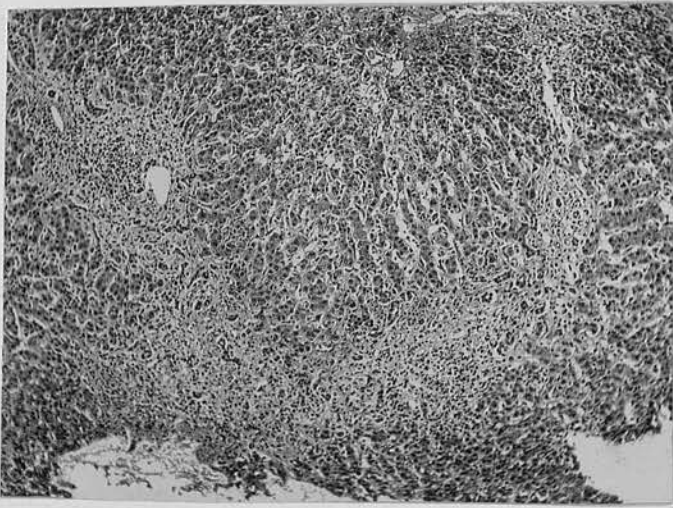
H. & E. X 60.

FIG. 39.

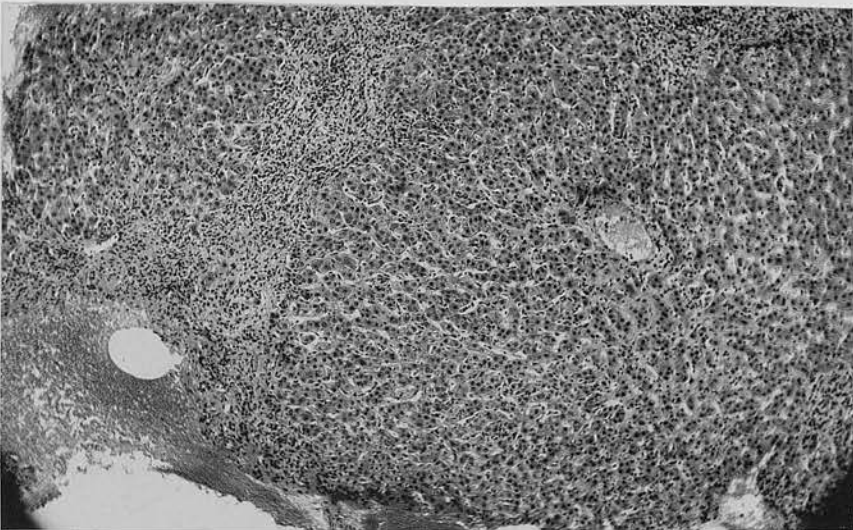
Case 10. Biopsy 2.

(Eight weeks later). There is an apparent shrinkage in the periportal inflammatory zone with less numerous bile ducts. The lobular inflammation has subsided.

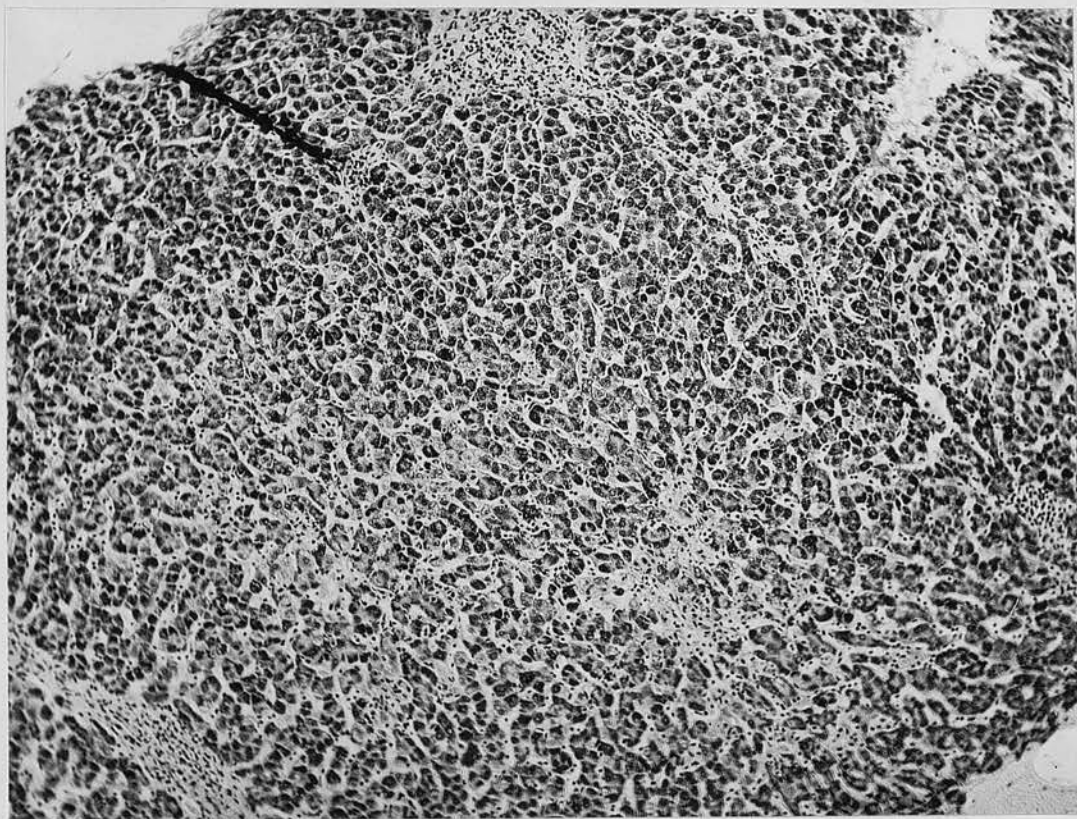
H. & E. X 60.



38.



39.



40.

Case 11.

Epidemic hepatitis. Healing zonal lesion. Scanty lymphocytic infiltrations in the portal tracts. Some disappearance of liver cells in the central zones.

Stained Best's Carmine. X 90.

The retrogressive hepatic zonal lesion is illustrated in figs. 38 and 39.

Case 10.- A man, aged 32, suffering from epidemic hepatitis. Jaundice had started 4 weeks before admission. This cleared up in 9 days, only to recur 7 days before he entered hospital. At this time, when the first biopsy was taken (fig. 38), the bilirubin was 6.4 mg. per 100 ml. The jaundice subsided very slowly during the next 6 weeks. The last biopsy (fig. 39) was performed 8 weeks after the first, and 12 weeks after the initial attack of jaundice. The serum bilirubin was now 0.6 mg. per 100 ml. At this stage clinical recovery appeared complete.

The first biopsy (fig. 38) shows a wide zone of periportal cellular connective tissue with numerous new bile-ducts. The lobule also shows some diffuse inflammatory change becoming more intense towards the centre. The last biopsy (fig. 39) shows a somewhat narrower periportal fibrotic zone with less numerous bile-ducts. The lobular inflammation has subsided.

Case 11.- A woman aged 24, suffering from infective hepatitis. 28 days jaundiced. Liver not palpable. Spleen just tipped. Serum bilirubin 2.2 mg. per 100 ml.

The section (fig. 40) shows scanty cellular mainly lymphocytic infiltrations in the portal tracts. The central zones show some disappearance of liver cells. The liver structure is otherwise normal.

This case is another example of the healing hepatic lesion often encountered when biopsy is delayed beyond the second week of jaundice.

4. Chronic residual and fibrotic lesions.- It is difficult to separate these clearly from the group of late zonal lesions just described. The last biopsy in case 10 illustrates clearly the periportal fibrotic scarring which may persist after clinical cure is apparent. Judging from the extreme rarity of true perilobular cirrhosis in routine autopsies, it seems likely that this type of residual scarring may ultimately disappear altogether. The question of progressive fibrosis will be discussed later.

Histological Detail

It is not proposed in the present paper to go into the histological minutiae, but for the sake of completeness we may summarise the main features of the changes found:

(a) Hepatic lobules.- In the severe cases, including all the lesions described as diffuse, there has been severe disorganization of the architecture of the hepatic lobules. The columns of liver cells broken up; the individual cells are separated and show decisive evidence of necrosis and autolysis. Many of the cells are swollen, often with large nuclei or with more than one nucleus, while others, in becoming necrotic, are changed into intensely eosinophilic masses, usually devoid of a nucleus. The cytological changes in the mixed zonal forms are less striking, and in the latter are limited mainly to a swelling and autolysis of cells round about the central vein and some apparent loss of cells in the region occupied by the infiltration round the portal tracts. In most cases dual and occasional multiple nuclei are common in the liver cells. Mitoses are seen but are less common than might be expected. Inclusion bodies have not been found in the hepatic cells

(b) Central zones.- The areas about the central veins usually show well-marked rarefaction due to the disappearance of liver cells (fig.43). The remaining cells often look bloated, with rarefaction of the cytoplasm and swollen nuclei. The wall of the central vein may undergo hyaline thickening and the entering sinusoids are dilated and much more readily seen than normally.

(c) Portal zones.- There is a marked tendency for accumulation of cells in these areas. Less noticeable in the severe acute lesion, they are of especial prominence in the milder early acute lesions. The cells are mostly small mononuclear cells of the histiocyte class, but a variable number of polymorphs is present and eosinophils may also be seen. The infiltrations tend to spread from portal tract to portal tract round the periphery of the lobules, and

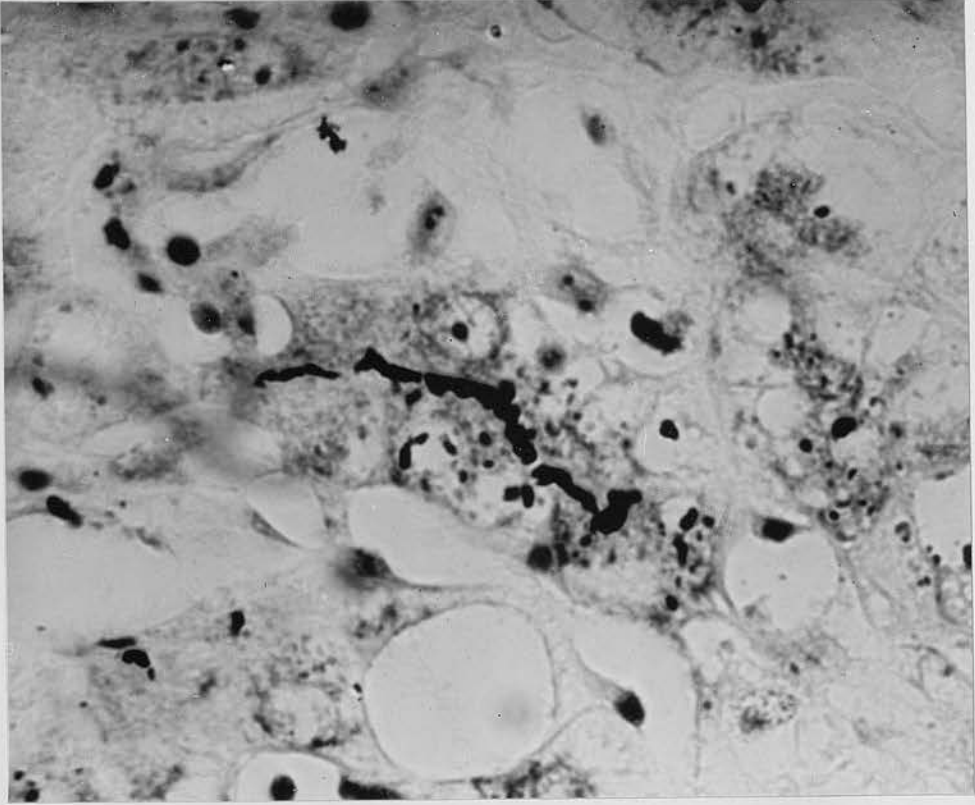
may send prolongations into the lobules in certain cases. The question arises whether these are simple infiltrations which push aside the tissues, or whether to some extent they replace necrotic liver cells. There is little exact evidence on this point, but since in some of the earliest cases these sites show a condensation of reticulin, suggesting some collapse of this portion of the lobule, it seems probable that liver cells have disappeared from these areas. Sometimes recognisable liver cells are seen trapped among the periportal cellular tissue. In cases of long standing the portal infiltrations show fibroblasts and new formation of young fibrous tissue.

(d) Sinusoids. - There is a general enlargement and hyperplasia of the sinusoidal endothelial cells giving the liver lobule an abnormally cellular appearance. Here and there these cells form small focal accumulations. It is possible that these are associated with focal necroses. Polymorphs leucocytes and eosinophils may also be seen in the lumina of the sinusoids.

(e) Glycogen. - The glycogen content of the surviving liver cells is usually well preserved. It disappears with cell autolysis. Odd cells, especially in the severer cases, appear abnormally heavily loaded with glycogen, apparently a necrobiotic phenomenon.

(e) Fat. - The Bouin fixed osmic acid stained sections showed a notable absence of stainable fat in the liver. In this respect the biopsy material differs from what is commonly found at autopsy.

(g) Reticulin. - The reticular framework of the lobules is often quite unexpectedly well preserved. When there is much hepatic cell loss - for example, in the collapsed central areas of badly damaged liver lobules - there is a condensation of reticulin and the same appearance is seen in the infiltrated portal zones. In the residual fibrotic lesions of the portal zones an increase in reticulin is seen which is followed by an increase in collagen.



41.

Arsenotherapy jaundice. Bile thrombi.
Methylene blue x 1100.

(h) Bile-ducts.- In rather over a third of the cases proliferation of bile-ducts was observable in the portal tracts. This occurrence could not be related to the duration or severity of the disease. Rather surprisingly it was less often seen in the more purely zonal type than in the more severe types (Note their retrogression in fig.38 compared with fig.39). In one fatal case in which the biopsy was made on the fourteenth day of the illness and death took place three days later, with the findings usual to subacute necrosis, the sprouting of the bile-ducts was more marked than in any other case in the series (fig.45). On the other hand, in many of our most severe non-fatal lesions, with duration of from 2 - 19 days, this change is not a prominent feature. In the chronic cases, in which a cirrhosis is developing, bile-duct proliferation is usual, although it may be obscured by the newly formed fibrous tissue.

(i) Pigment.- Some bile-staining of the central cells of the lobule, many of which are in process of necrosis, is common and was a constant feature. Many Kupffer cells were also bile-stained in the central zone. "Bile thrombi" were often seen; occasionally they were prominent (fig.41). The distribution of the thrombi was chiefly in the mid-zones of the lobules; the larger interlobular ducts appear normal.

(j) Iron.- The Prussian-blue reaction does not show any abnormal quantities of iron in the liver.

(k) Haemorrhages.- The alcohol-fixed material is not the best in which to observe small haemorrhages, but, with this proviso, these have not been found.

HISTOGENESIS AND PROGRESS

It is impossible to give accurate data about the duration of the lesion because we are not able to fix with certainty the time of commencement of the disease. This difficulty is well illustrated by the following cases.

Case 12.- A woman, aged 24, suffering from epidemic hepatitis. There was a history of a previous attack when aged 9 years. She was admitted with headache and dizziness of seven days' duration. There was neck-rigidity, suggesting meningeal irritation, but no

jaundice. Lumbar puncture gave a normal cerebro-spinal fluid. Mild jaundice appeared four days after entry when a liver biopsy was performed. The serum bilirubin was then 2.5 mg. per 100 ml. Four days later it had fallen to 0.6 mg.

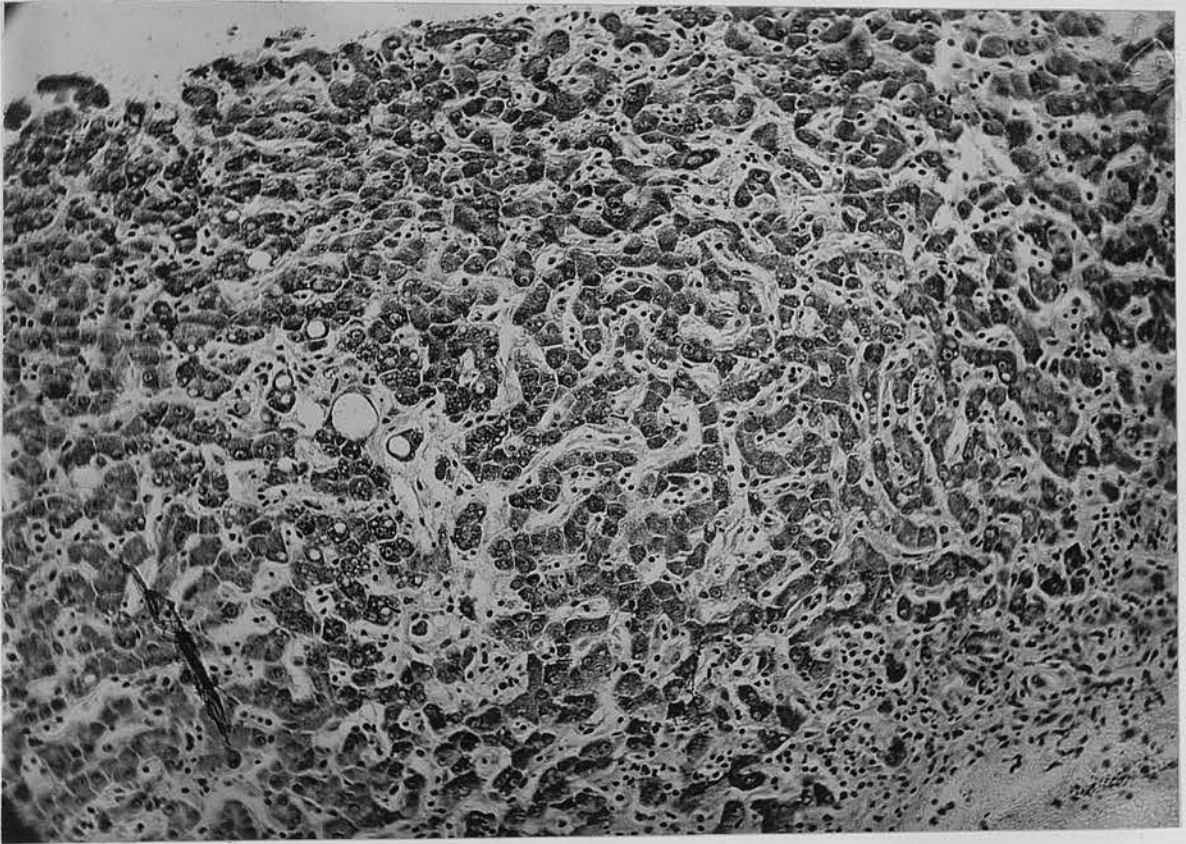
The biopsy specimen showed a well-marked portal zonal lesion: there was some bile-staining of the central cells and bile thrombi were found. Polymorphonuclear leucocytes were seen in the periportal infiltration, but early bile-duct proliferation was found and the lesion was considered, on pathological grounds to be of a subacute or early chronic nature. It is impossible that these changes could have developed on the day the icterus was noticed, and it seemed probable that the hepatitis was present for at least the whole period of the patient's illness.

Case 13.— A 58 year old man was in hospital under observation for pulmonary tuberculosis, chronic bronchitis and cor pulmonale. He was also having arsenotherapy for syphilitic glossitis. Hepatomegaly and a very slight icteric tinge of the sclera drew attention to the liver. The serum bilirubin was 2.5 mg. per 100 ml. Liver biopsy was performed. Four days later the serum bilirubin was 1.4 mg. per 100 ml. Seventeen days after the first biopsy, before discharge to a Sanatorium, a second liver biopsy was performed.

The first biopsy (fig.42) shows an active hepatitis with conspicuous liver cell necrosis and focal infiltrations. Portal zonal lesions are much in evidence and show bile duct proliferation. The surviving liver cells show a normal complement of glycogen and some contain a moderate amount of fat. The lobular pattern is not lost. Reticulin stains show a good pattern with some reticulin condensation in the region of the portal tract infiltrations.

The second biopsy (fig.44) shows good recovery.

These two cases are of great interest. In both liver sections showed a fairly severe active hepatitis. In both jaundice was minimal and transient. If the patient had not been under observation, the one for the prodromal symptoms, the other for an unrelated



42.

Case 13. Biopsy 1.

An active hepatitis with conspicuous cell necrosis and focal infiltrations. Surviving liver cells show a normal complement of glycogen and some contain a moderate amount of fat.

Shin + meyer

Case 13. Biopsy 1.

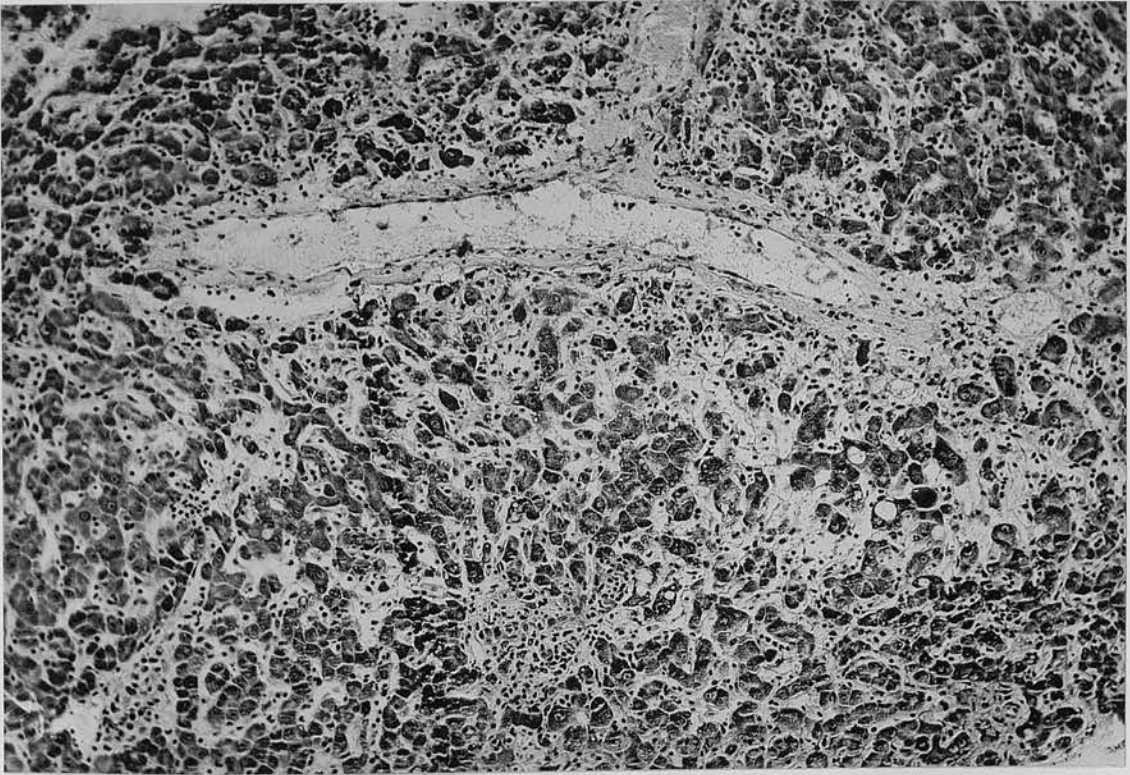
Longitudinal section of central vein showing adjoining liver cell necrosis and inflammatory change.

Stained Best's carmine. X 130.

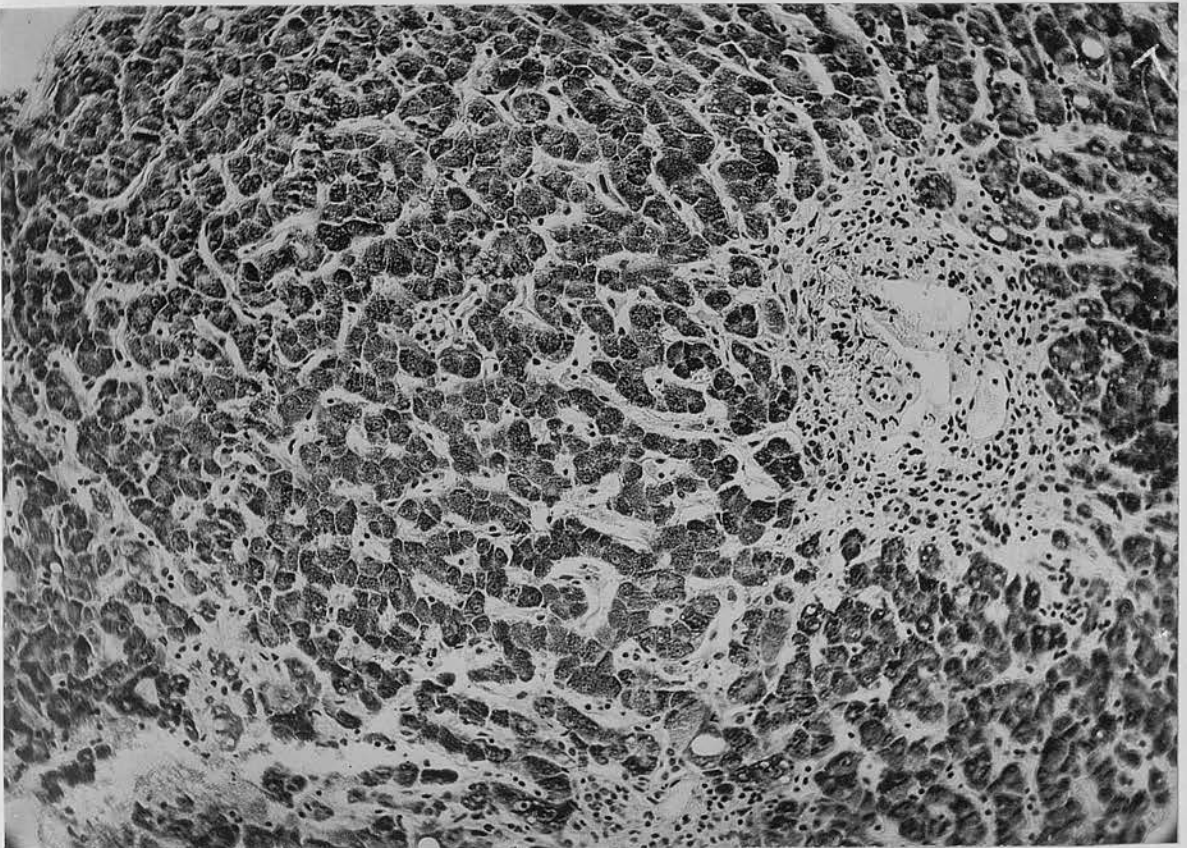
Case 13. Biopsy 2.

Recovery with residual portal scarring.

Stained Best's carmine. X 165.



43.



44 .

condition, the hepatitis would not have been diagnosed.

The findings in these and other early cases have convinced us that the liver inflammation probably begins with the prodromal symptoms which are so often regarded as gastro-intestinal in origin.

Looking at our cases broadly, it appears that the acute and mild case of up to a week's duration is likely to show a lesion which is in the main limited to the portal zone, with but little destruction of the liver lobule. An acute and severe case is likely to show diffuse or mixed type of lesion with well-marked disorganisation of the lobular architecture, with marked degenerative changes in the hepatic cells and considerable histiocytic and leucocytic infiltration of the whole lobule.

A survey of the total case material studies makes it evident that degeneration and autolysis are most severe in the early stages about the hepatic vein and that the leucocytic and histiocytic reaction may proceed from the portal zones centrally.

The further evolution of the hepatitis will be discussed in the next section.

Discussion.

Our material is in accord with the mass of evidence pointing to hepatitis as the pathological basis of epidemic jaundice, (Eppinger 1922; Nordmann 1925; Klemperer, Killian & Heyd, 1926; Schrumpf 1932; Gaskell 1933; Barber & Osborn 1939; Roholm & Iversen 1939; Fox et al 1942; Siegmund 1942; Cameron 1943; Lucké 1944). The old view of Virchow (1865) that the jaundice was due to obstruction of the ampulla of Vater by a plug of mucus need no longer be considered.

Intubation studies have shown no evidence of duodeno-biliary catarrh (Van Rooyen & Gordon 1942) or of biliary obstruction (Hayman & Read 1945).

The mechanism of production of icterus is a matter of some interest. The disruption of the liver-cell columns with their intercellular bile canaliculi may create a form of obstruction to bile flow. The isolation of liver cells may also lead to the excretion of bile into the surrounding tissue spaces.

It is also obvious that the degree of damage may be such that the surviving liver cells are quantitatively inadequate to excrete the bile pigment brought in the blood-stream. Histologically, pigmented cells are seen especially at the centre of the lobule. Some of these are obviously necrotic, while others have lost their glycogen. The latter may accumulate bile because they are unable to excrete it, while the former become pigmented in consequence of their death. The affinity of dead tissue for bile is a common pathological phenomenon. Further out in the lobule we meet with "bile thrombi" (fig 41), the finest inter-cellular canaliculi being filled with accumulated bile which has obviously failed to escape. There is an absence of evidence of any bile stasis in the inter-lobular branches of the bile-ducts, suggesting that, if any obstructive factor exists, it is intralobular.

The material studied has consisted of hospital cases which may be more severe than many milder cases treated at home. Our clinically mildest and most transient cases, however (cases 12 & 13) had very significant liver lesions. A striking feature has been the remarkable severity of the pathological picture. Several of the cases seem to have been verging on acute liver necrosis - 21 out of 87 cases showed histological damage of more than 50% of liver cells - yet the normal course is to complete recovery. As we shall see fatal or permanent damage is rare.

In company with other workers no histological difference has been found between the liver lesion in epidemic hepatitis and arsenotherapy jaundice (Roholm & Iversen 1939; Mitchell 1943, Dible & McMichael 1943) or serum jaundice (Fox et al 1942, Ministry of Health 1943; Findlay et al 1944; Lucké 1944; Hayman & Read 1945). In general however the serum and arsenotherapy cases fell into the severer grades. Of 21 cases with at least 50 per cent liver cell damage 15 were arsenotherapy or serum jaundice cases.

The histological picture of intrahepatic biliary obstruction described in arsenotherapy jaundice by Hanger & Gutman (1940, and also mentioned by Chaikin & Chlenoff (1945) has not been seen in our series.

However our cases usually occurred during the second course of arsenical injections whereas those of the previous authors resulted after 1-3 injections and may represent an entirely different syndrome.

THE COURSE OF ACUTE HEPATITIS

The general pathology of the liver in acute hepatitis has been described. Chapter 12 will deal with general course of the disease with special reference to pathological changes in the liver. Some biochemical connections will be established.

The general progress of acute hepatitis may be summarised as follows -

1. Death
 - a) Acute liver necrosis.
 - b) Subacute liver necrosis.
2. Clinical, histiological and biochemical recovery with or without residual scarring in the portal tracts.
3. Development of a hepatic cirrhosis
 - a) Active.
 - b) Latent.

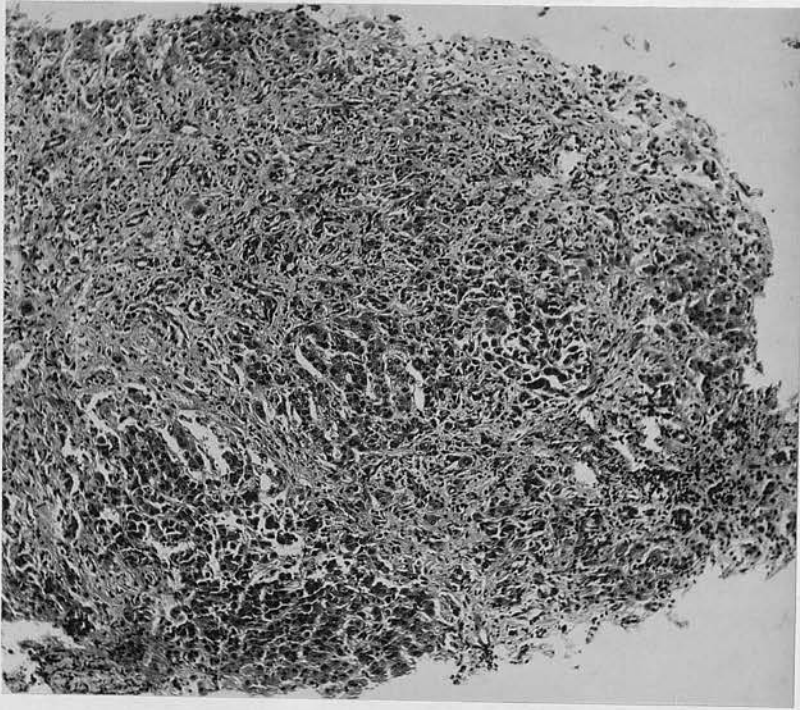
In addition a group of cases have been encountered in which symptoms, possibly referable to the liver, develop after hepatitis. These have been designated "the Post Hepatitis Syndrome" and will also be discussed.

Fatal Acute Hepatitis.

This is a rarity. The mortality for recent epidemics is summarised in table 27 below.

THE MORTALITY OF ACUTE HEPITIS IN RECENT EPIDEMICS

Author	Date	Place of Epidemic	Type of Hepitis	No. of cases.	Deaths per cent.
United States	1942	United States Army	Yellow fever serum jaundice.	28,585	0.216
Fox et al.,	1942	Brazil	"	979	2.55
Mitchell	1943	Canadian Army	(a) Arsenotherapy jaundice.	-	1.47
			(b) Infective hepatitis.	-	0.94
Ministry of Health Memo.	1943	England	Measles serum.	37	21.0
Oliphant et al.,	1943	Virgin Isles	Yellow fever serum jaundice.	1039	0.
Findlay et al.,	1944	West Africa	"	689	0.145
Turner et al.,	1944	United States Army.	"	4083	0.34
Hayman & Reed.	1945	United States Army.	"	405	0.25



45.

Case 14.

Arsenotherapy jaundice, There is extensive fibrous change with the liver cells, many of which are degenerate, split up into islets.

H.E. X 90.

The exact number of cases occurring during this last war remains a military secret. It seems, however, that the mortality is usually less than half of one per cent. In our series of 89 cases there were three deaths. This high mortality rate is due to the more severe cases being hospitalised while the milder examples were treated at home, or occurred in unselected army groups.

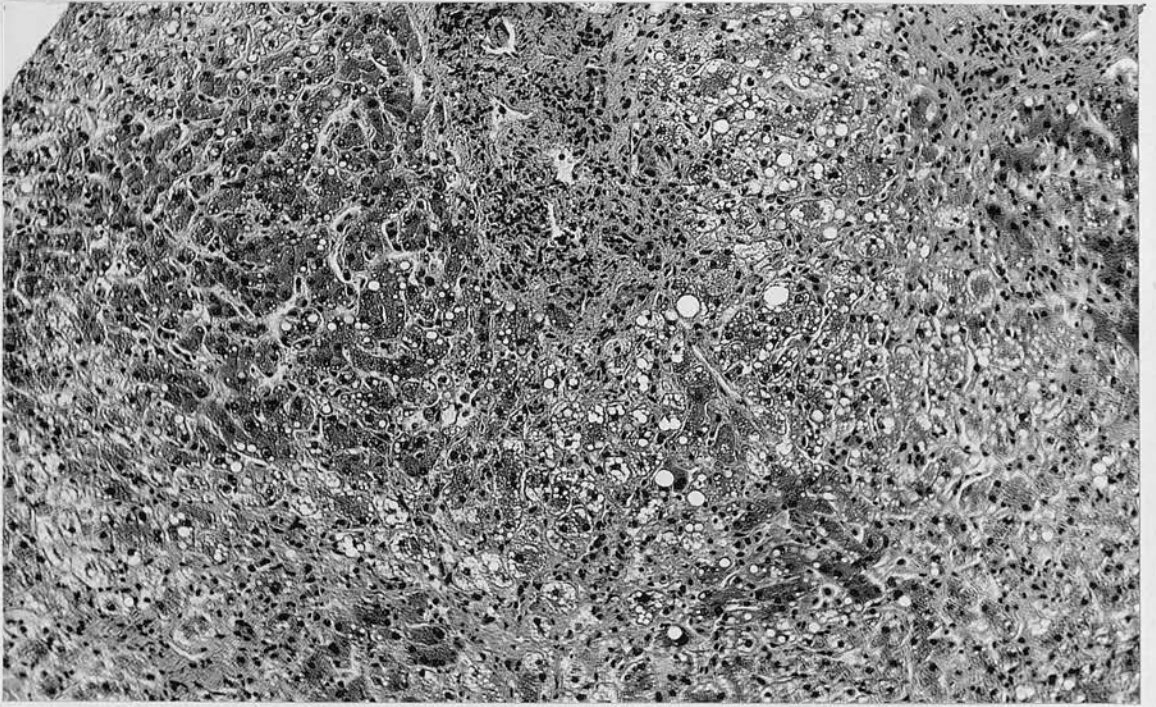
It has long been noted that fatal cases of acute hepatitis at autopsy show the changes described in idiopathic "yellow" atrophy (Cockayne, 1912, Bormann, 1940, Fox et al., 1942. Siegmund, 1942, Findlay et al, 1944). An excellent description of 125 fatal cases has recently been published by Lucké (1944a), all showed acute necrosis of the liver. The pathological changes are related to the duration of disease. The liver may be rapidly overwhelmed by the infective process. The picture is then that of acute destruction of liver cells. Case 4 is an example of such a condition. More often the process is of longer standing, there is commencing regeneration of liver cells proceeding side by side with the necrosis, and the lesion acquires the characteristics of subacute liver necrosis.

Case 14. A man aged 63 had been receiving arsenotherapy for neurosyphilis. He had been jaundiced for 14 days, icterus was progressive. 10 days previously the abdomen distended with fluid and he became drowsy.

The hepatic biopsy section (fig.45) showed gross distortion of the liver. Microscopic islets of commencing nodular hyperplasia are also seen.

The patient became comatose and died 48 hours after the biopsy. At autopsy a typical picture of subacute necrosis was seen in the liver.

Case 15. A 54 year old woman had been receiving arsenical treatment for tertiary syphilis. Jaundice was noticed 6 weeks before admission. After 2-3 weeks the icterus lessened only to deepen 1 week before admission. At this time intractable vomiting started and the day of entry into hospital she relapsed into coma. The liver was palpated 5 cm.



46.

Case 15.

Arsenotherapy jaundice. The picture is that of a subacute necrosis. Nodular hyperplasia of remaining liver cells. Other hepatic cells replaced by ghost like outlines. Some fatty changes in the necrosing liver cells. In the lower centre area, the necrosis of a portal tract, with excessive fibrous tissue and proliferation of bile ducts can be seen.

H.E. X 120.

below the right costal margin. Free fluid could be detected in the abdomen. The patient appeared moribund and died 2 hours later.

Aspiration liver biopsy was performed 10 minutes postmortem. Sections (fig.46) show gross destruction of the liver. Small groups of surviving liver cells undergoing nodular hyperplasia can be found, but these are very wide apart. Some of the cells show fatty change. Glycolysis is complete. The intervening area is occupied by a matrix consisting of ghost-like outlines of degenerate liver cells, fibroblasts, proliferating bile ducts and inflammatory cells many of round cell type. The lobular structure of the liver is completely lost. The reticulin framework is disintegrated.

DISCUSSION.

For the study of liver necrosis, tissue obtained during life or immediately postmortem is essential. The part played by postmortem autolysis in the production of the "classical" picture of subacute "yellow atrophy" has recently been described by Van Beek and Haex (1943). These authors believe that the extent of many of the changes described have occurred after death. This is also suggested by Roholm and Iversen (1939). We have confirmed this work, not only in liver necrosis where postmortem changes occur particularly rapidly, but in other conditions. A true histological picture of the liver can only be given by material obtained by biopsy done during life. Material obtained within an hour of death is fairly satisfactory. Obviously this proviso is particularly applicable to the tropical medicine.

TABLE 28.

Case No.	Duration of jaundice days	Sex	Age	Serum Bilirubin mg/100ml.	Serum Phosphatase mg/100ml.	Serum Proteins g/100ml.				Serum Cholesterol mg/100ml.
						Total	Alb.	Glob.	A/G.	
4	3	M	27	10	11	5.8	2.8	3.3	0.93	180
14	14	M	63	14	11	5.2	2.8	2.4	1.1	192
15	42	F	54	16	16	5.0	2.0	3.0	0.66	110

The biochemical findings in the three fatal cases

The biochemical findings in the three fatal cases are tabulated above. The very high serum bilirubin in two cases, the conspicuous serum protein changes and in particular the serum albumen of less than 3 g per cent suggest an unduly severe hepatitis. Serum phosphatase and serum cholesterol are not remarkable.

The reason why one case of hepatitis goes on to rapid complete recovery while another passes to acute necrosis and death is not known. Clinically ominous signs are increasing great depth of jaundice and the development of ascites. The development of drowsiness and coma usually precedes a fatal termination.

The General pattern of histological restitution.

The reticulin framework of the liver is usually intact even amidst the most extensive disorganisation of the liver cell columns. It seems probable that this framework of reticulin provides a scaffolding on which the liver cells regenerate into the normal lobular pattern. Once recovery starts it proceeds extremely rapidly. The central areas are the best to reform. Portal tract infiltrations disappear slowly.

Material. The details of the recovery process in the liver will be illustrated by descriptions of nine cases in which liver sections were obtained at the acute stage and at various intervals during the period of recovery.

The results of the biochemical procedures discussed previously will be included and will serve to amplify the statements made in part I.

Recovery from acute Diffuse Hepatitis.

Case 1. This case demonstrates the usual mode of recovery from a severe lesion. The extreme degree of hepatitis has been described above (fig.22). Even at this stage however the reticulin framework of the liver was virtually intact. (fig.23).

The patient made a rapid recovery and in three weeks the serum bilirubin had fallen from 11.9 mg/100 ml. to 1.6 mg/100 ml. and five weeks later the serum bilirubin was 0.6 mg/100 ml. At this stage a further biopsy was taken. (fig.24). This shows almost complete recovery. The lobular pattern is normal. The liver cells have regenerated into regular columns right up to the central vein. The inflammatory cells formally seen throughout the section are absent. The only remaining abnormality is some cellularity in the portal tracts. The significance of this will be discussed later.

The next case illustrates rapid recovery from an even more severe diffuse lesion.

Case 3. A man aged 59 suffering from arsenotherapy jaundice. This case has also been mentioned above.

Serial hepatic punctures were performed to follow the progress of recovery.

Biopsy 1. Jaundiced 7 days. Serum bilirubin 14.0 mg/100 ml.

The liver sections have been described already (fig.25). The hepatitis was the severest encountered among the non-fatal cases. However reticulin stains show a remarkably good preservation of the reticulin framework (fig.26). There is a little thickening and condensation of reticulin in the region of the central veins.

Biopsy 2. 18 days jaundiced. Serum bilirubin 14.5 mg/100 ml. During the 11 days intervening between the two biopsies there has been a quite amazing degree of recovery (fig.27). The lobular pattern can now be readily identified. The liver cells have formed into regular columns. They possess an abundant glycogen content. The recovery has not proceeded right up to the centre of the lobule which still shows necrosis of liver cells and some infiltration with inflammatory cells. The portal tracts show reticulin condensation and contain proliferating bile ducts.

The conspicuous portal tract changes and the excessive initial severity of the hepatitis suggested that this case might proceed to permanent structural liver damage. Further biopsies were undertaken.

Biopsy 3. 32 days jaundiced. Bilirubin 2.6 mg/100ml.

Sections show further recovery of liver structure. (fig.28). The liver cells have now regenerated right up to the central vein. There is still conspicuous extra tissue in the portal tracts but this is less than in the previous biopsy. This tissue contains proliferatory bile ducts (fig.29) fibrous strands can also be seen passing from the portal tracts towards the centres of the lobules. There is a tendency for linkage between adjoining portal tracts. These fibrous changes suggest a progression towards cirrhosis. It was therefore agreed that before discharge from hospital a final biopsy should be undertaken.

Biopsy 4. 61 days jaundiced. Serum bilirubin

0.5 mg/100 ml. There is no evidence of cirrhosis (fig.30). The lobular architecture is normal. Histiocytes have proliferated throughout the liver infiltrating in both sinusoids and portal tracts. There is a tendency for focal accumulation of the histiocytes in the sinusoids. Portal tract fibrosis is slight, the bile duct proliferation evidenced on previous occasions is not now noticed. The centres of the lobules show a little reticulin condensation (fig.31) and the reticulin framework is on the whole heavier than usual.

This section was interpreted as histiocytic proliferation with residual portal scarring. The histiocytic proliferation is believed to be scavenger response to the products of the necrosis of liver cells.

The laboratory findings are graphed (fig.47).

47.

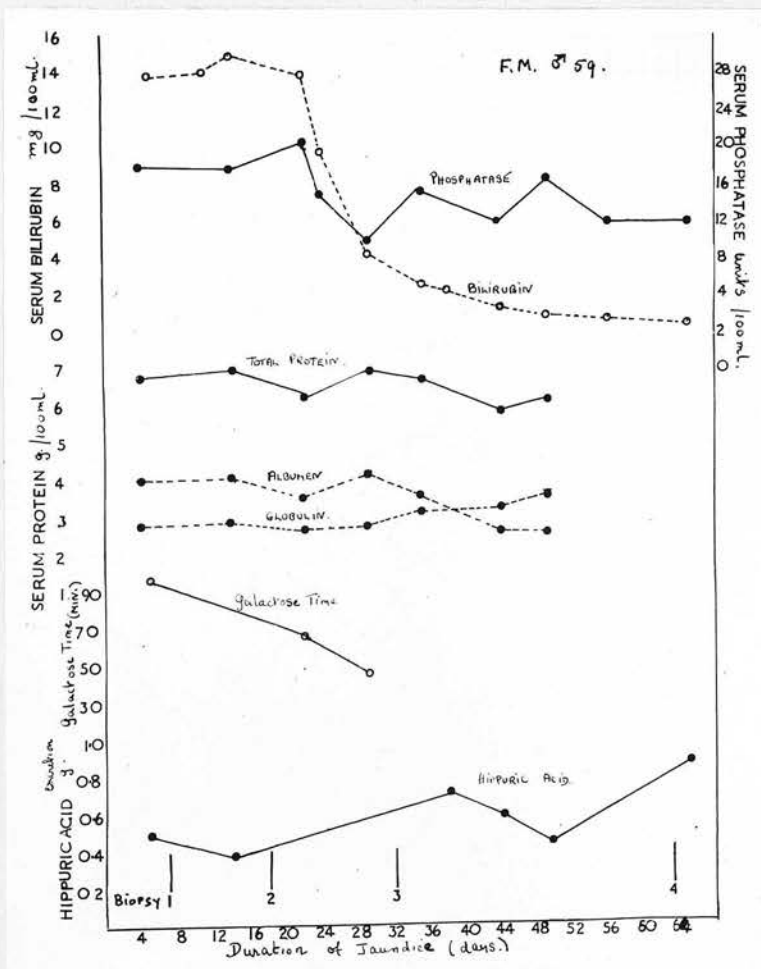


FIG. 48.

Case 16. Biopsy 1.

Severe diffuse hepatitis.

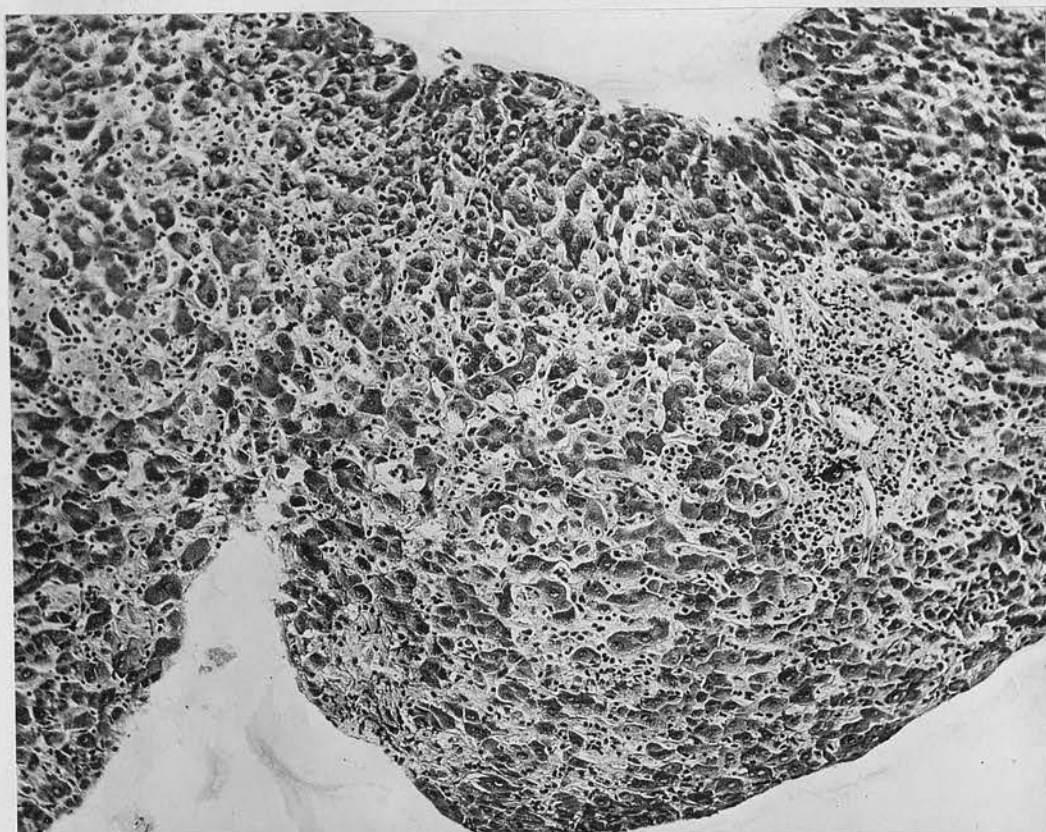
Best's carmine stain. X 130.

FIG. 49.

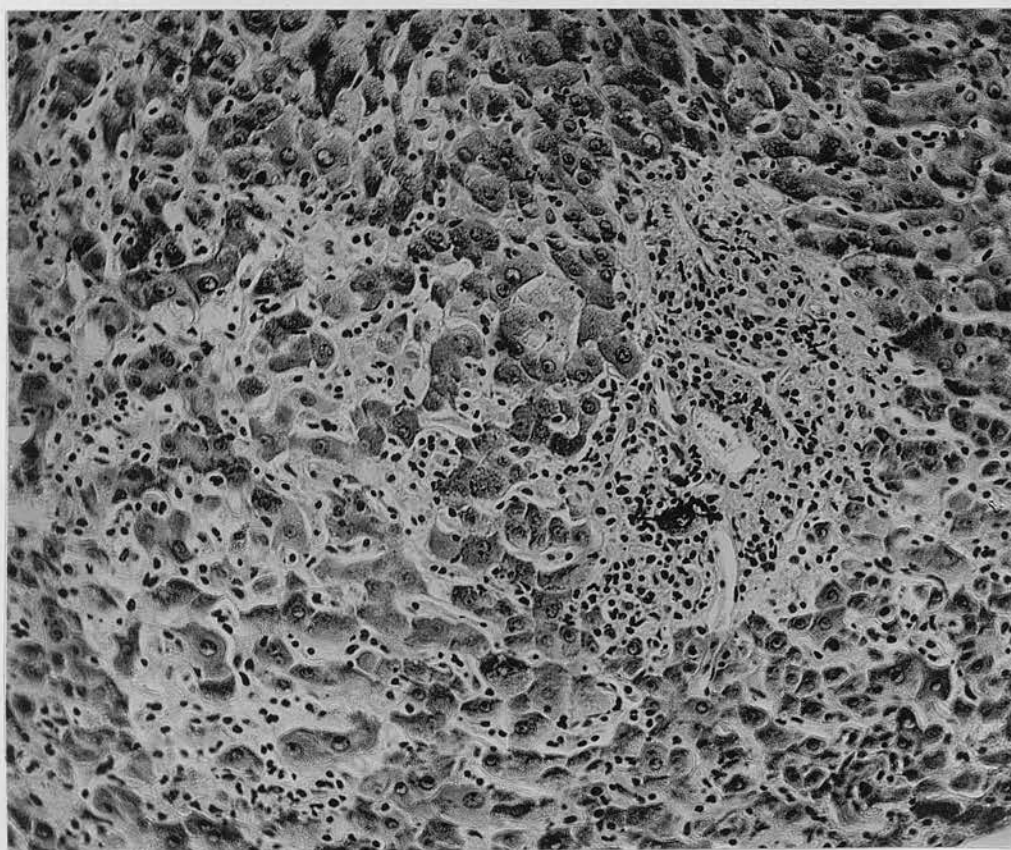
Case 16. Biopsy 1.

Higher power view showing the disappearance of liver cells many of which remain only as faint outlines. The portal tract is infiltrated with round cells. L

Best's carmine stain. X 205.



48.



49.

FIG. 50.

Case 16. Biopsy 2.

Considerable recovery. Focal necroses remain.
Recovery has not proceeded right up to the
central vein.

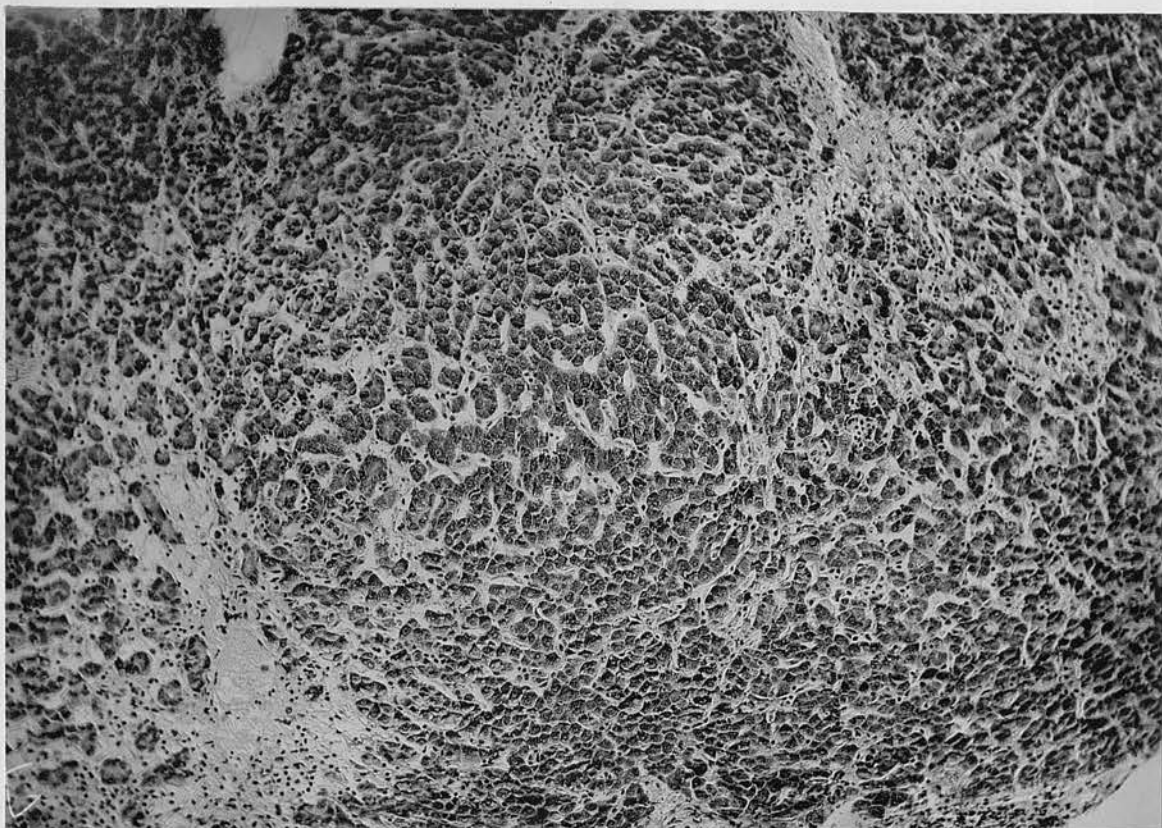
Best's carmine stain. X 105.

FIG. 51.

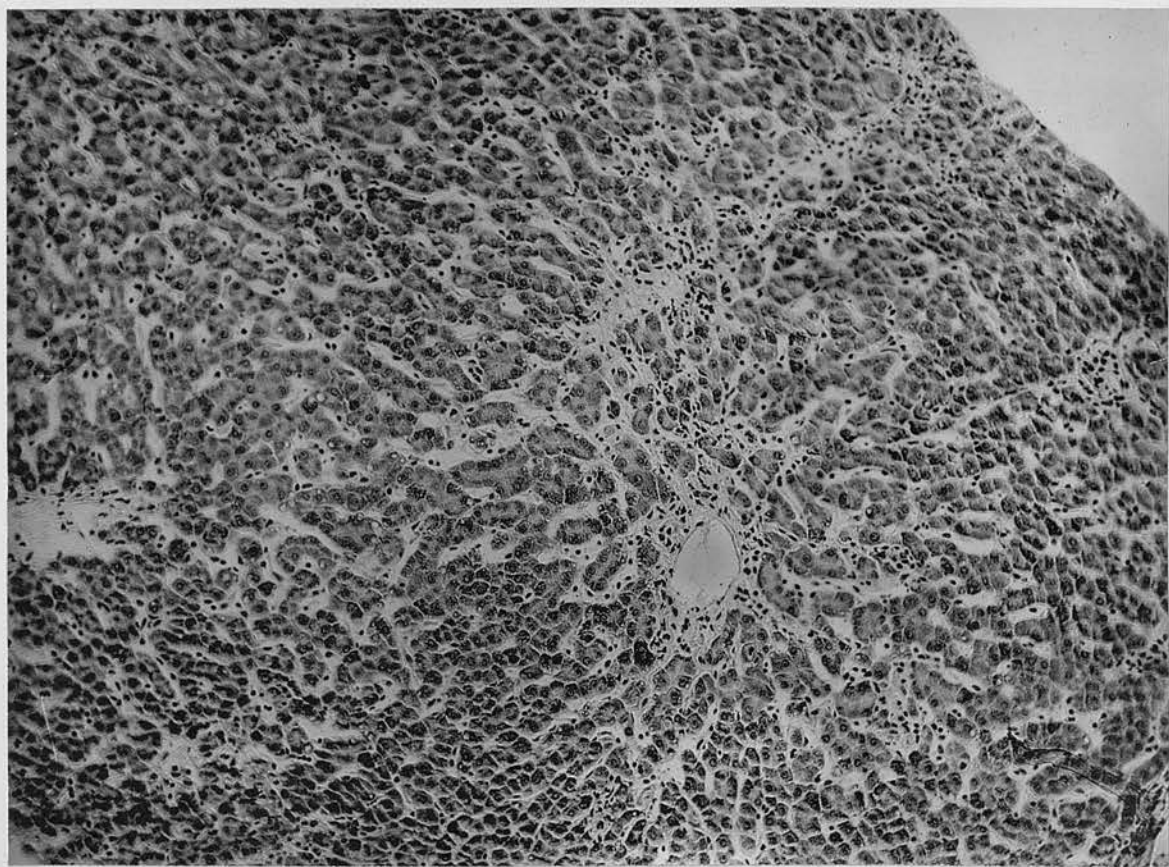
Case 16. Biopsy 3.

Further recovery. Histiocytic proliferation
in sinusoids. One small focal necrosis.

Best's carmine stain. X 135.



50.



51.

FIG. 53.

Case 17. Biopsy 1.

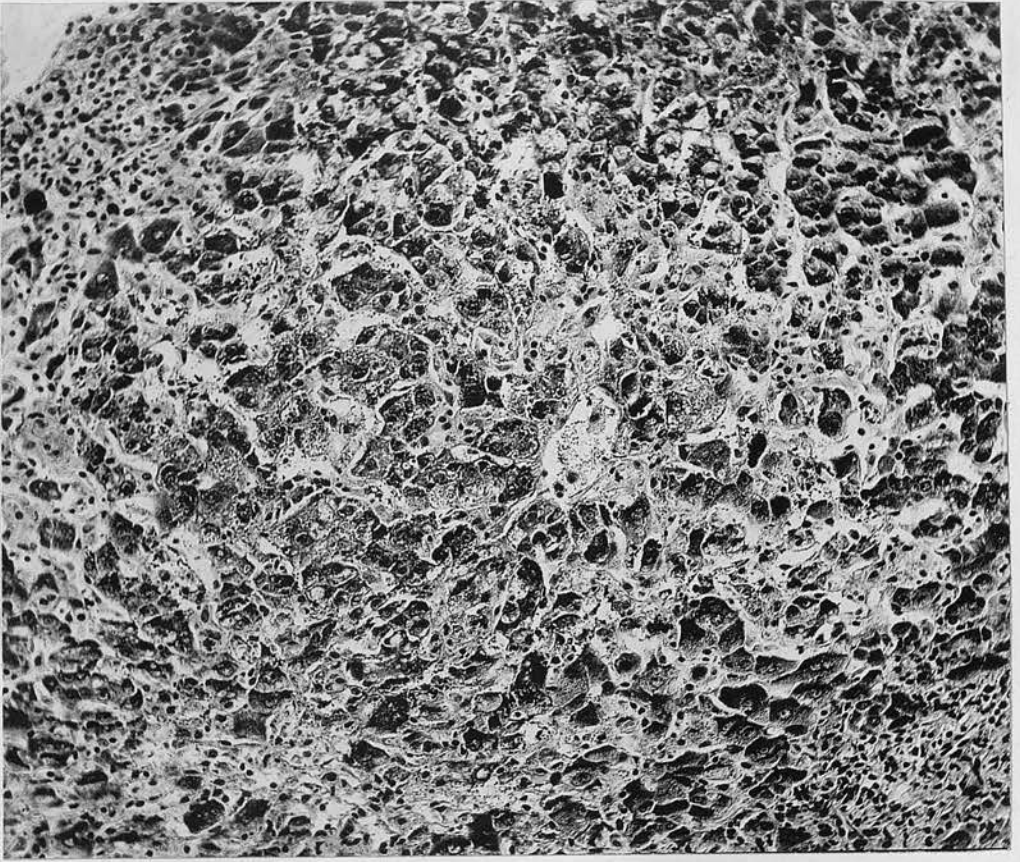
Severe mixed hepatitis. Note patchy glycogen staining. The portal tracts are highly cellular and many of the cells are polymorphs. Best's carmine stain. X 160.

FIG. 54.

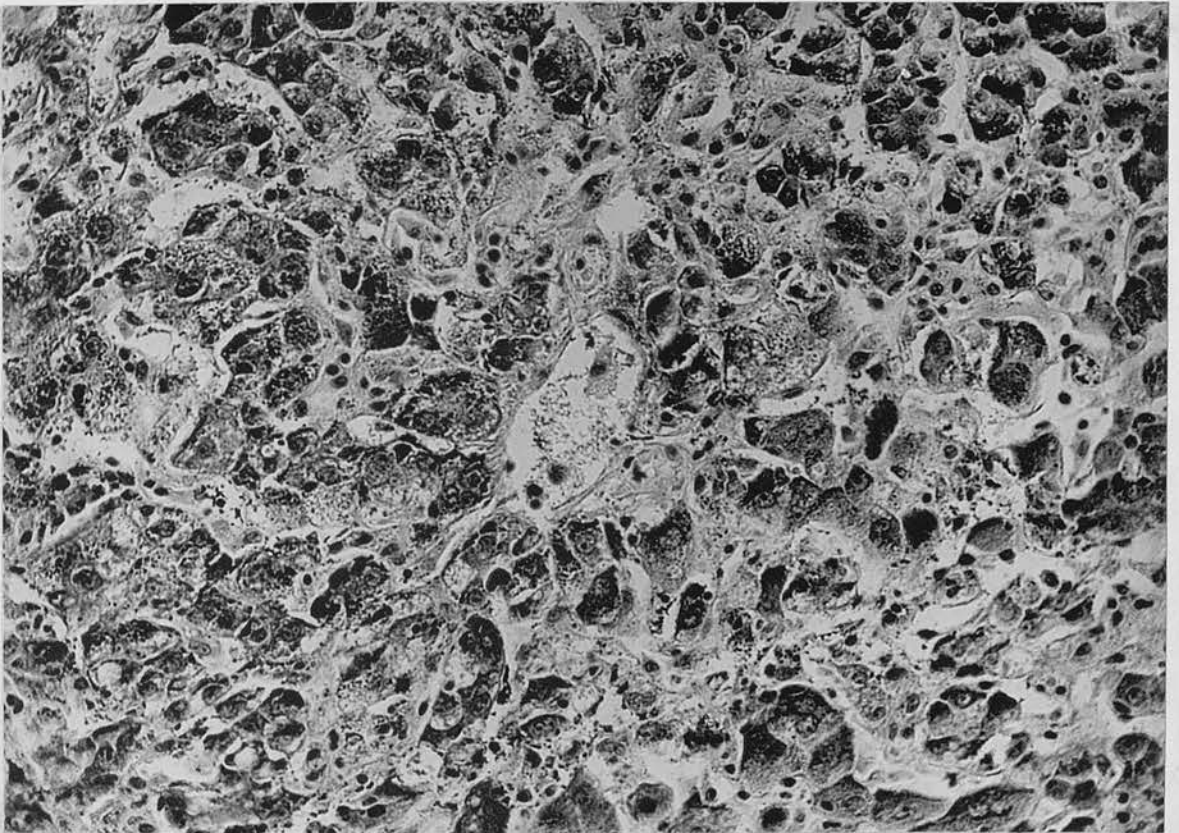
Case 17. Biopsy 1.

Same area more highly magnified. The patchy distribution of glycogen and the disappearance of liver cells around the central vein are well demonstrated.

Best's carmine stain. X 280.



53.



54.

FIG. 55.

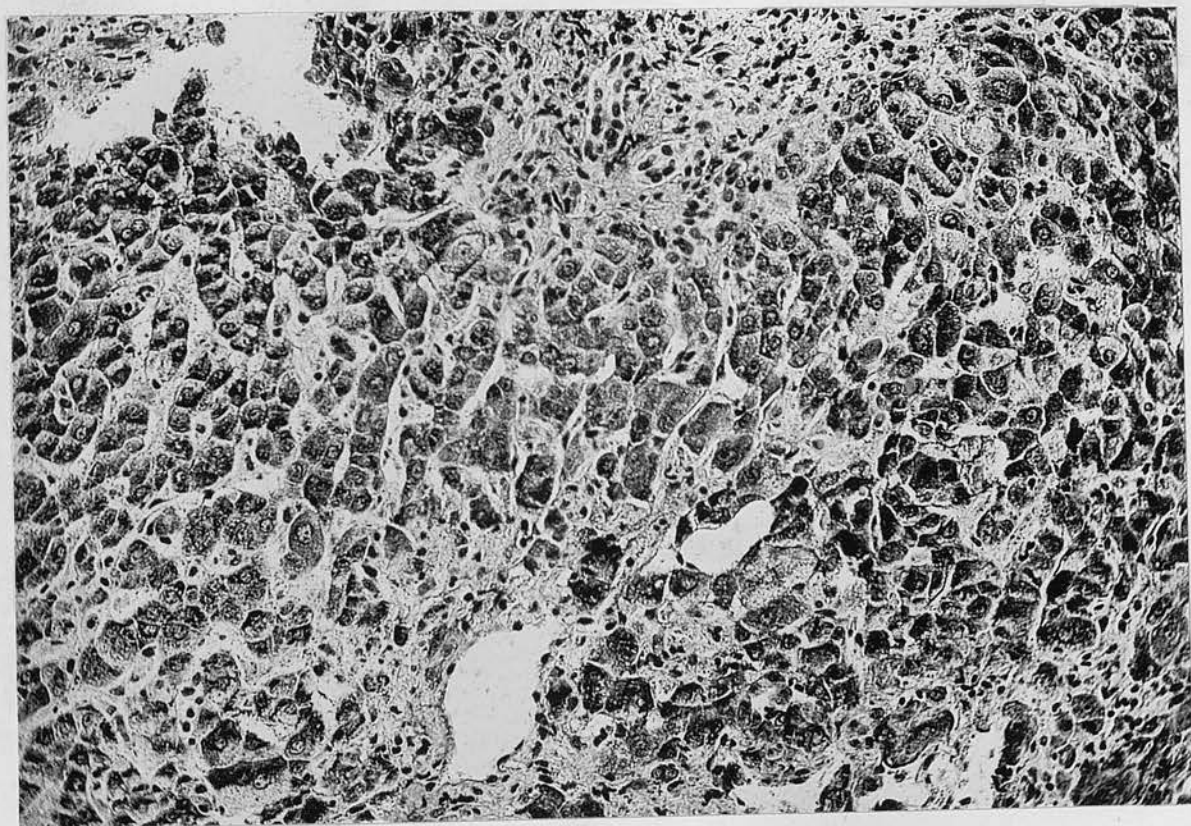
Case 17. Biopsy 2.

Considerable recovery of liver structure.
Central autolysis of liver cells is still seen.
The portal tracts contain many cells but these
are now round celled.
Best's carmine stain. X 220.

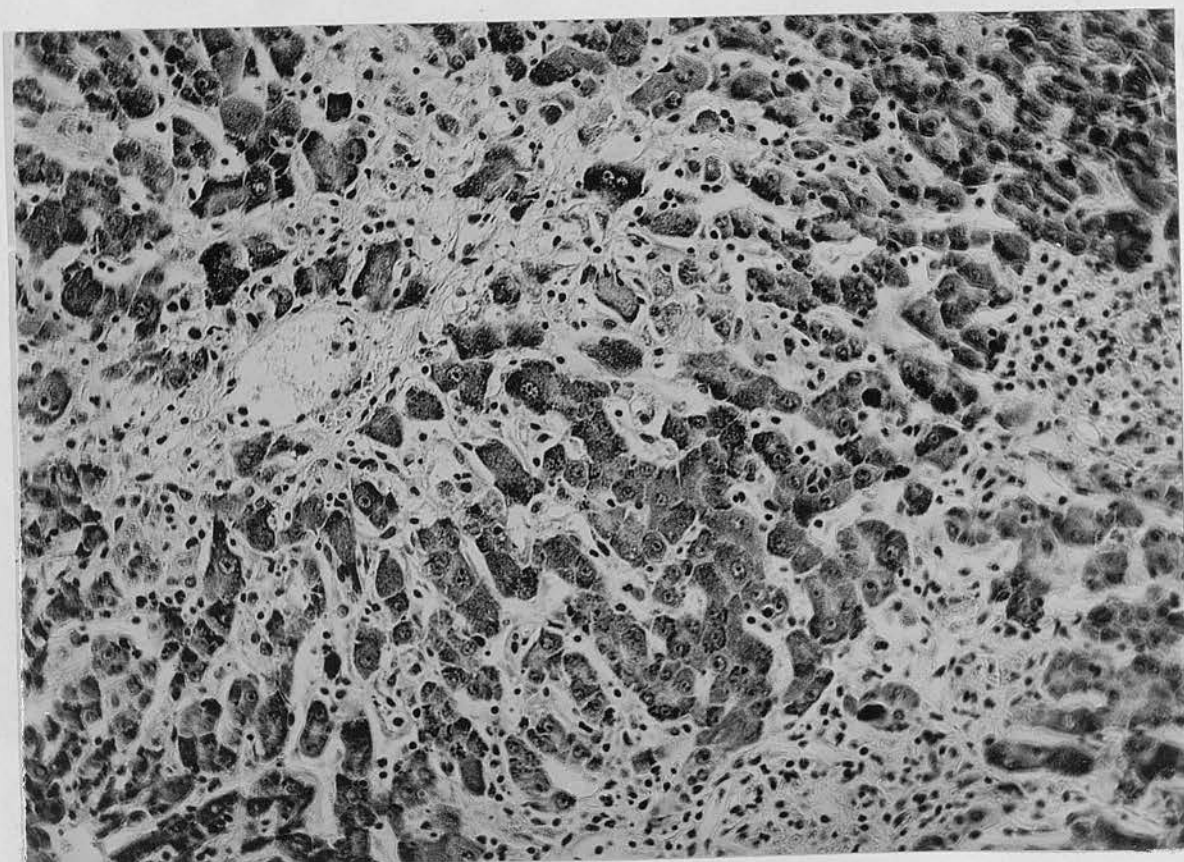
FIG. 57.

Case 18. Biopsy 1.

A severe zonal and diffuse hepatitis. There
is a tendency towards dissociation of individual
liver cells.
Best's carmine stain. X 220.



55.



57.

FIG. 59.

Case 19. Biopsy 1.

Mixed diffuse and zonal hepatitis with conspicuous central autolysis and portal tract infiltrations.

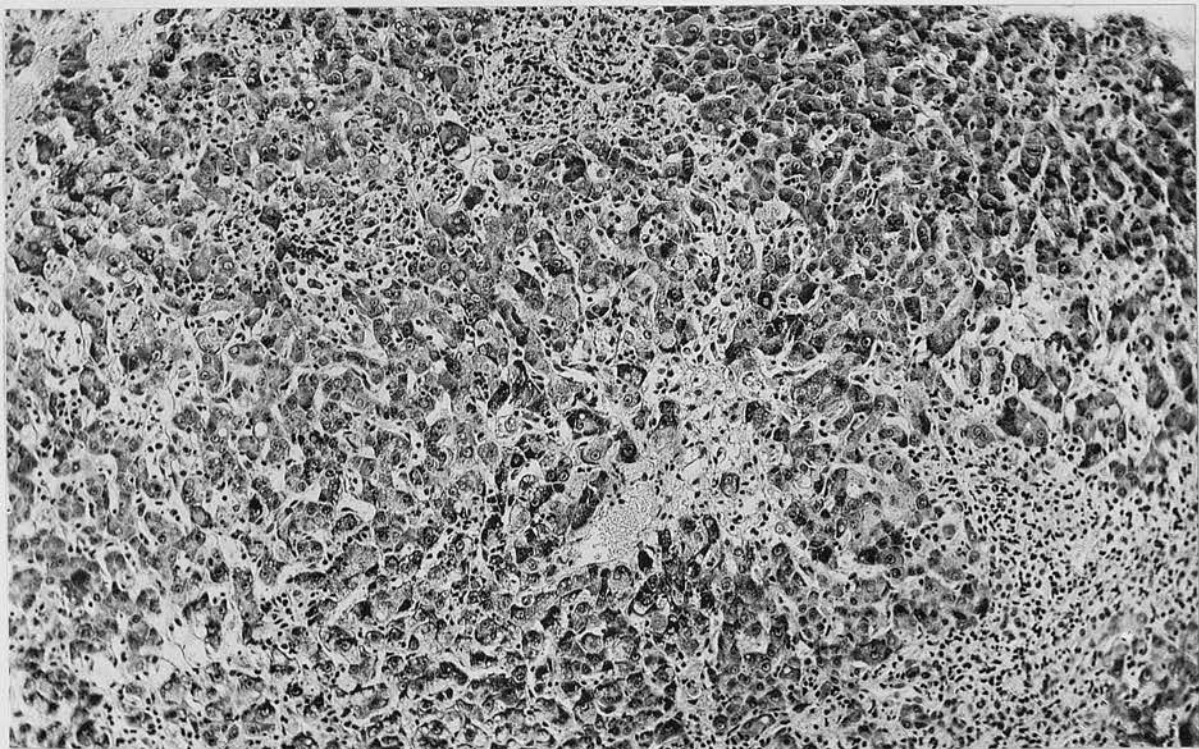
Best's carmine stain. X 120.

FIG. 60.

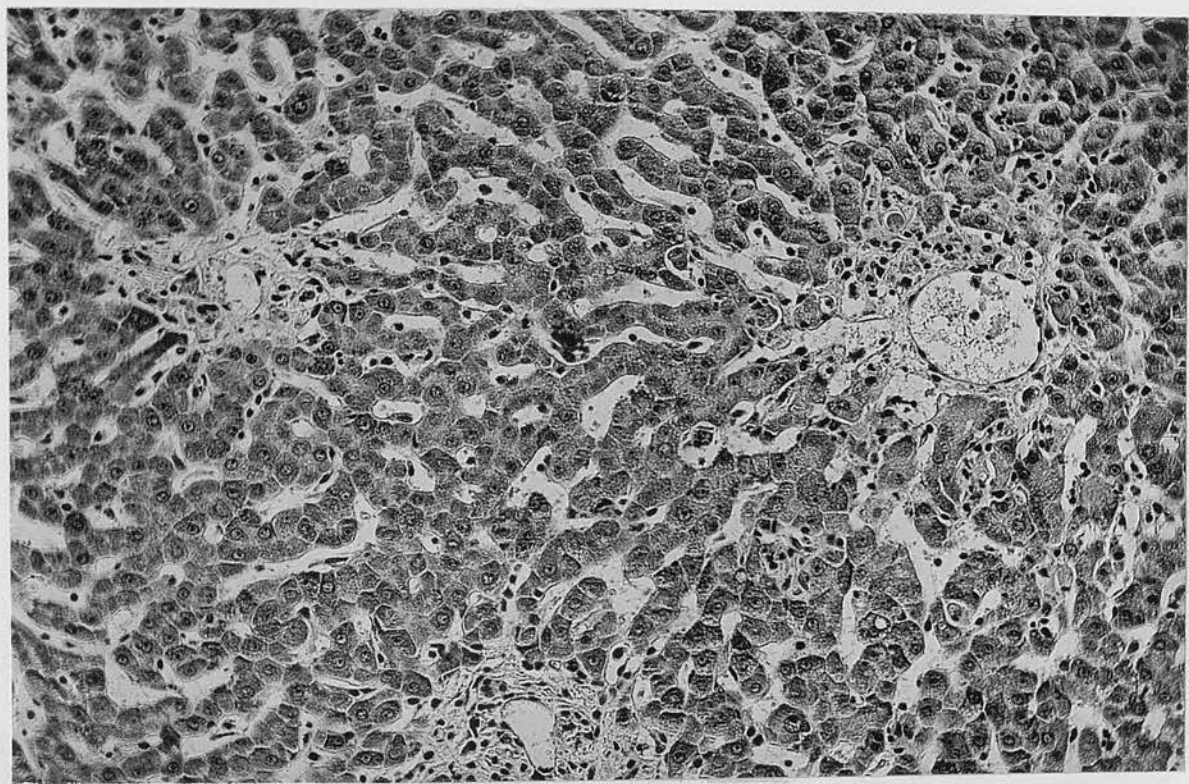
Case 19. Biopsy 2.

Histological recovery. Some central rarefaction and a very slight excess of tissue in the portal tracts remain.

Best's carmine stain. X 190.



59.



60.

FIG. 62.

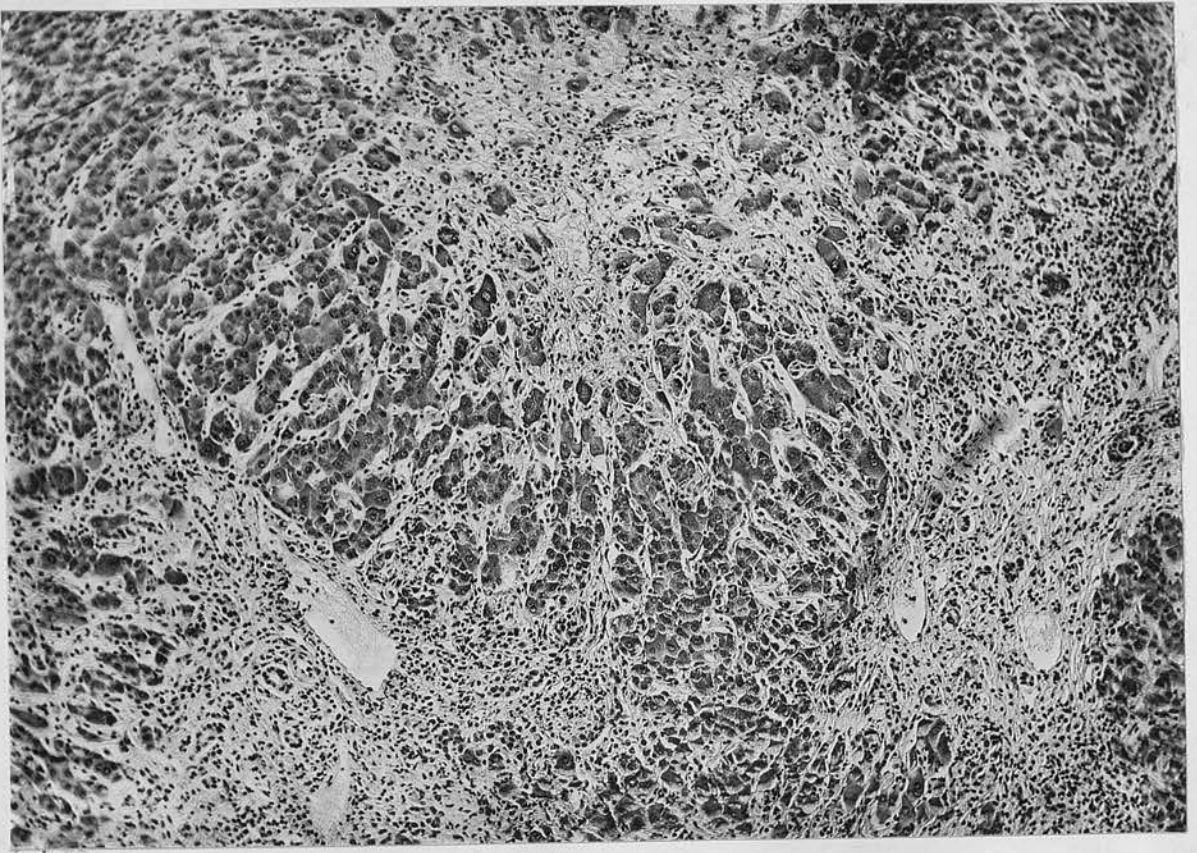
Case 20. Biopsy 1.

Severe zonal hepatitis. Central autolysis of liver cells. Conspicuous portal tract infiltrations with bile duct proliferation. Best's carmine stain. X 105.

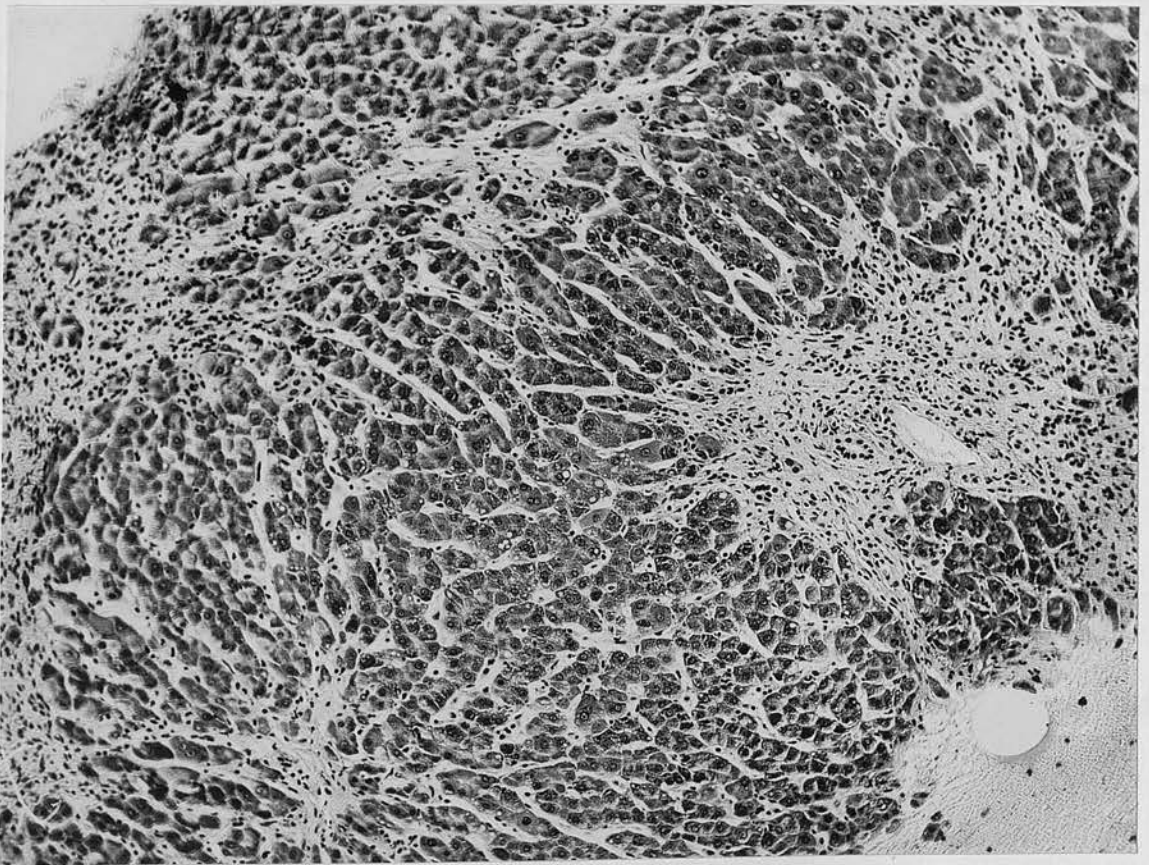
FIG. 63.

Case 20. Biopsy 2.

Considerable recovery. Scattered areas of fibrosis remain especially in the portal tracts. Best's carmine stain. X 120.



62.



63.

At the outset the high serum bilirubin, the conspicuous serum protein changes and the impaired galactose tolerance all suggest a severe lesion. Rapid fall in serum bilirubin level followed quickly on regeneration of liver tissue the later fall to normal was slower. Normal galactose tolerance was observed at the time of liver biopsy 2. when evidences of hepatitis were still noted. The results of the hippuric acid synthesis test did not correlate with liver histology.

This case demonstrates the degree and speed of recovery possible from the most severe hepatitis. Pathological sequelae do not necessarily follow such a lesion. The patient has been seen 15 months after the first biopsy. He is in excellent health and doing a hard day's work as a bus conductor.

The last case in this group describes the recovery from a less severe diffuse lesion.

Case.16. A 41 year old soldier suffering from arsenotherapy jaundice. Jaundice had been present for 9 days. The liver was enlarged to the umbilicus, the serum bilirubin was 4.3 mg/100 ml.

Sections show a fairly severe diffuse hepatitis (figs.48 and 49). For the next five days the patient was very ill. Anorexia, vomiting and right upper abdominal pain persisted. The temperature occasionally rose to 101° F. The serum bilirubin rose to 11.8 mg/100 ml. Symptoms then subsided and on the 22nd. day of jaundice the serum bilirubin had reached 2.5 mg/ 100 ml. A further biopsy was performed.

Biopsy 2. Sections show considerable recovery (fig.50). Areas of focal necrosis of liver cells can be seen but the liver otherwise appears normal.

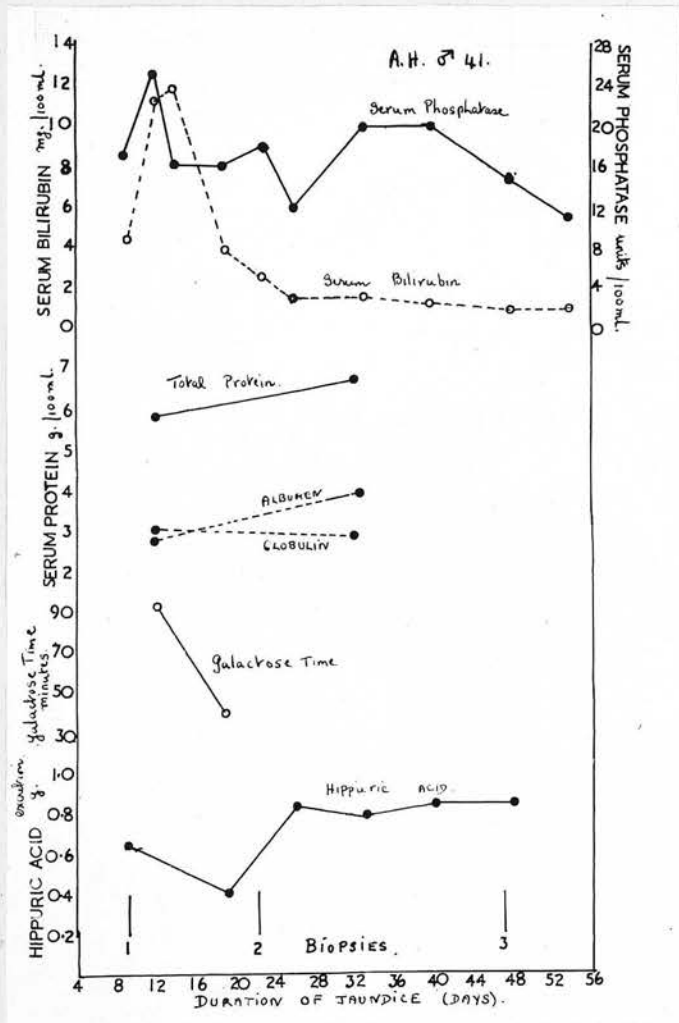
Further clinical recovery was uninterrupted and by the 47th day of jaundice the serum bilirubin was 1.9 mg/ 100 ml. A third biopsy was undertaken.

Biopsy 3. Sections show complete restitution of normal liver structure (fig.51). Portal tracts are normal. There is a very occasional small focal necrosis. The histiocytic proliferation noted in

the previous case is also seen in lesser degree.

The biochemical findings in this case are graphed (fig.52).

52.



The rapid initial fall of serum bilirubin as the liver recovers and the bile canaliculi are reconstituted is well seen. The changes in serum bilirubin bear no relation to the changes in serum phosphatase. The serum albumen was initially less than 3 g/100 ml. This bespeaks a severe hepatic lesion. The recovery of the proteins with a change from a reversed to a normal albumen/globulin ratio is seen. As in case 3 normal galactose tolerance returned before histological recovery was completed.

Recovery from "mixed" zonal and diffuse hepatitis.

The general pattern of recovery is similar to that described for the diffuse lesion. Portal tract changes are more prominent and there is a higher incidence of cases with residual portal tract scarring after clinical recovery.

Case 17. A soldier aged 23 suffering from arsenotherapy jaundice. Jaundice had been present for 4 days. Serum bilirubin 11.5 mg/100 ml.

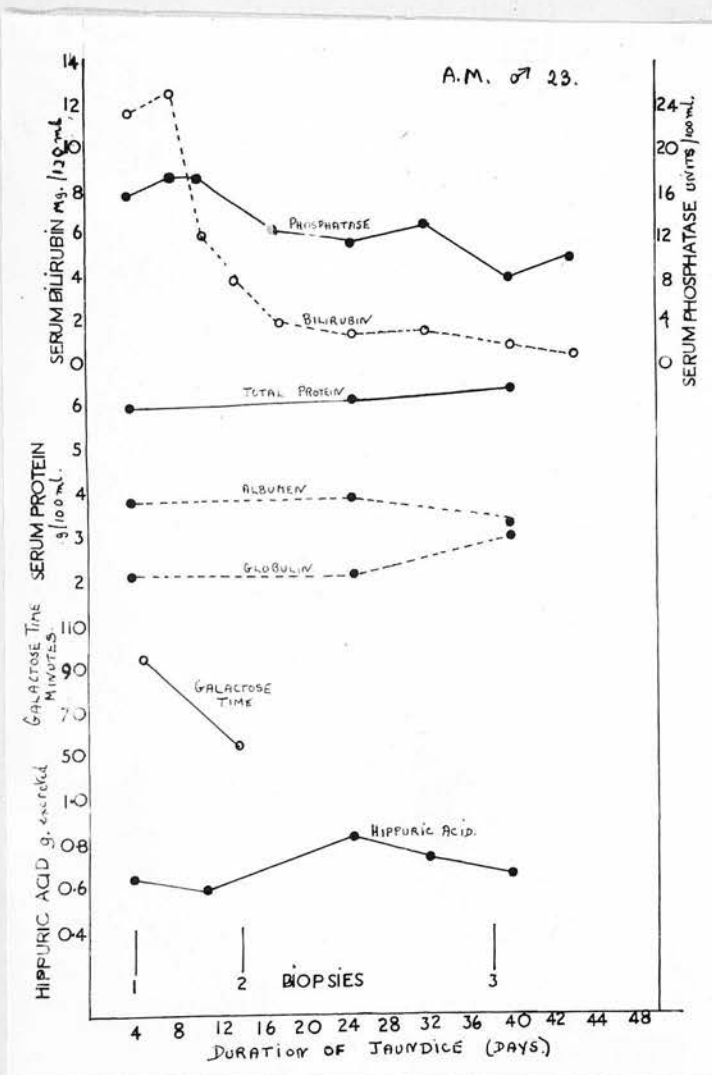
The liver section (figs. 53 and 54) show a fairly severe mixed hepatitis. A conspicuous feature is the patchy glycogen staining of the liver cells. Some cells contain little or no glycogen others contain excess.

The patient made a steady recovery and 10 days after the first biopsy the serum bilirubin was 4 mg/100 ml. A second biopsy was performed.

Biopsy 2. Sections show considerable recovery (fig. 55). Central autolysis of liver cells and portal tract infiltrations are still seen.

On the 38th day after the onset of jaundice the serum bilirubin was 1 mg/100 ml. A third biopsy was done and this showed disappearance of the acute changes. Portal tract fibrosis remained.

The biochemical findings are graphed (fig. 56) on next page.



The changes in the hippuric acid test could not be correlated with the histological picture. The patient was discharged with a test value lower than that recorded in normal subjects. This was the only biochemical abnormality revealed after clinical recovery. It seems doubtful whether the change could be attributed to the residual fibrosis in the portal tracts. The patient has remained well and was last seen 14 months after discharge from hospital.

The next case is also of severe mixed diffuse and zonal hepatitis.

Case 18. A soldier aged 33 suffering from epidemic

hepatitis. 10 days jaundiced serum bilirubin
18 mg/100 ml.

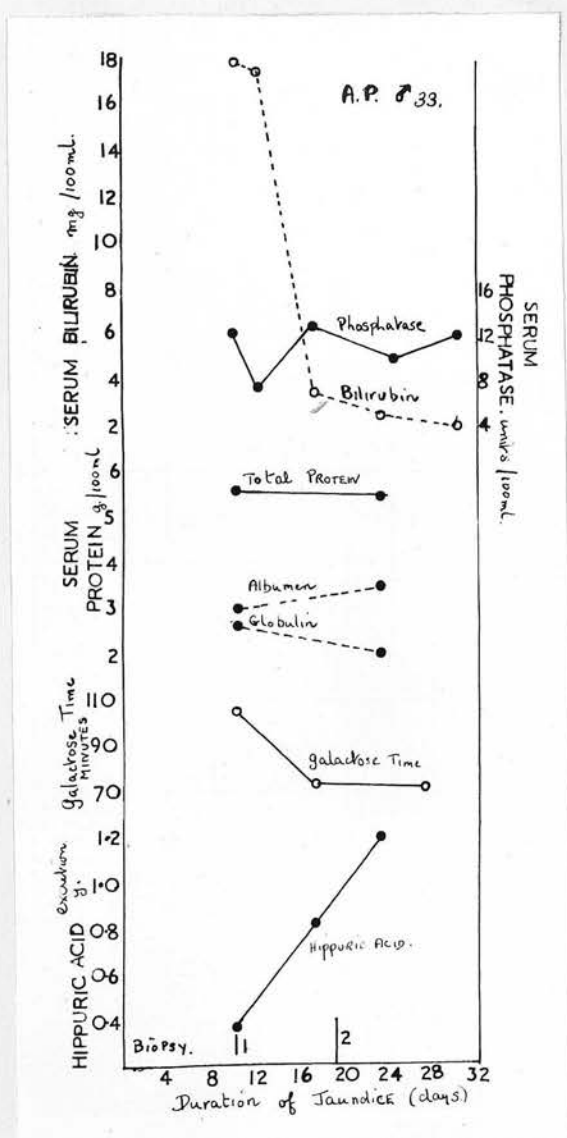
Biopsy sections show a very severe hepatitis (fig.57).. A conspicuous feature is the relative isolation of surviving liver cells. In places their margins are rounded and they appear dissociated one from another. This feature of cell dissociation in acute hepatitis has been noticed by other workers (Kirshbaum & Popper, 1940; Siegmund 1942). Portal tracts show the usual cellular infiltrations.

The patient made a rapid recovery and 9 days after the first biopsy the serum bilirubin had reached 3 mg/100 ml.

Biopsy 2. The liver cells now form regular columns. The central necrosis has shrunk and is represented by a small area of central rarefaction. The increased cellularity of the portal tracts is no longer obvious.

The biochemical changes are illustrated (fig.58).

58.



It is interesting to note that, although the second liver biopsy demonstrated incomplete recovery, the biochemical methods used all gave normal results.

This man has been seen 14 months after discharge from hospital. He remains well.

The degree of histological recovery demonstrated in the next case is much more complete.

Case 19. A 41 year old labourer suffering from arsenotherapy jaundice. Jaundiced 5 days. Serum bilirubin 8 mg/100 ml.

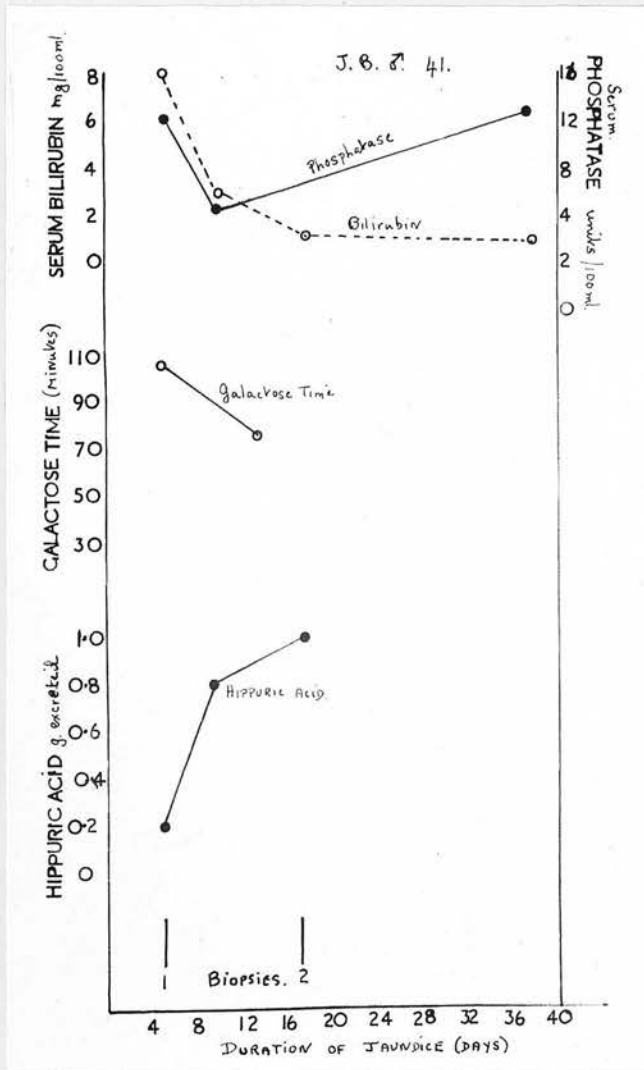
Sections show a moderately severe hepatitis. Central necrosis, bile thrombi and portal cellular

infiltrations are prominent (fig.59).

The patient made an uninterrupted recovery and 12 days after the first biopsy the serum bilirubin was 1.2 mg/100 ml. A second biopsy was undertaken. Biopsy 2. Sections show almost complete recovery of liver structure. There is very slight portal tract thickening and some remaining central rarefaction.

The biochemical findings are shown in (fig.61).

61.



Clinical and histological recovery coincided with return of normal biochemistry.

Recovery from Zonal Hepatitis.

The purely zonal hepatic lesion is usually encountered in the early mild case or when biopsy is delayed beyond the second week of jaundice and recovery has commenced. As will have been noticed many of the cases of "diffuse" and "mixed" hepatitis

described above have, during recovery, passed through an entirely zonal phase. Although a few cases are undoubtedly zonal from the outset, in the majority of instances, the lesion represents one stage in the progress from a diffuse lesion to complete recovery. The emphasis on the portal tract lesion suggests that it is in this group that residual portal tract scarring is most common.

Portal scarring after clinical recovery has been demonstrated in two of the previous cases (cases 10 and 13 figs.39 and 44). The residual liver change was not associated with symptoms referable to the liver, neither did we detect any abnormalities with the biochemical methods in use.

The next case demonstrates recovery from a severe zonal lesion.

Case 20. A 20-year old girl suffering from epidemic hepatitis. 12 days jaundice, liver palpable at the level of the umbilicus, serum bilirubin 6.9 mg/100 ml.

Sections show an extremely severe zonal hepatitis (fig.62). There is widespread central autolysis of liver cells and the portal tracts are heavily infiltrated with inflammatory cells. Bile duct proliferation is also seen. Passing between central and peripheral lesions the remaining liver cells lie in fairly regular columns. The sinusoids contain inflammatory cells of all types. Reticulin stains showed some condensation in the central areas and in the portal tracts, but on the whole good preservation of the reticulin framework.

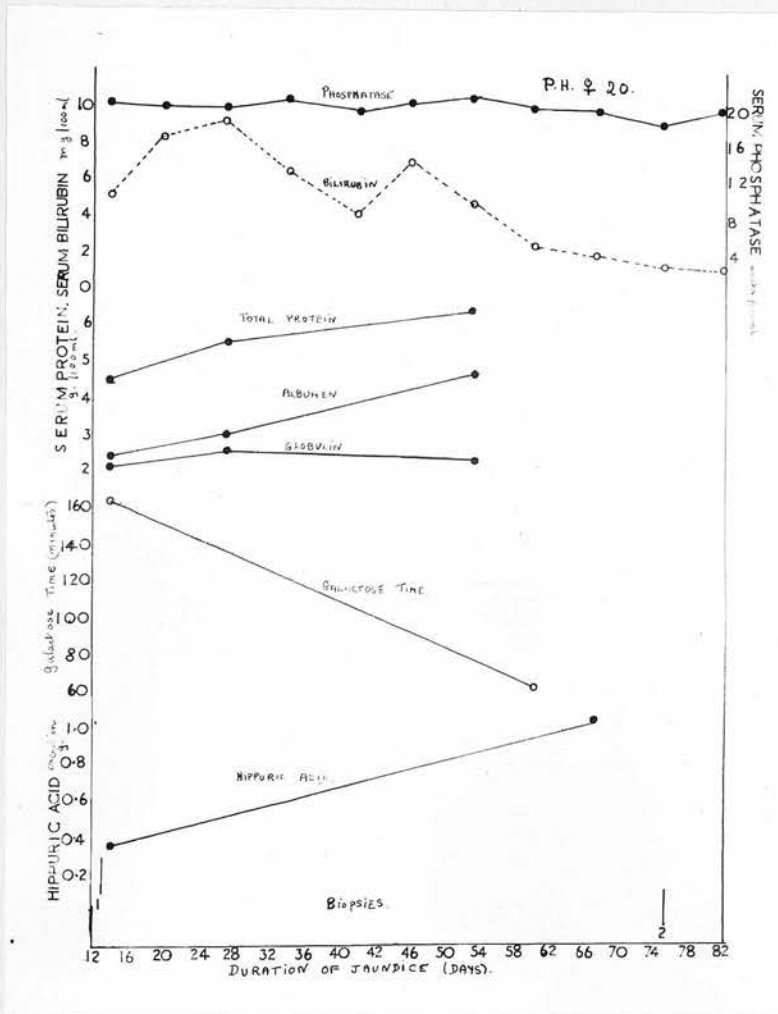
The patient made a slow recovery. There was an intermittent pyrexia of 103°. The ankles and sacrum showed pitting oedema. The abdomen became very distended, a fluid thrill could be elicited. Eventually all symptoms subsided, the jaundice faded and 9 weeks after the first biopsy the sacrum bilirubin was 1.4 mg/100 ml. and a second biopsy was undertaken.

Biopsy 2. Sections show considerable liver cell regeneration. The acute inflammatory cell reaction is no longer visible. Scattered areas of fibrosis remain, especially in the portal tracts. (fig.63).

The patient was discharged from hospital. She reported three months later, she felt well and had returned to factory work. Liver and spleen were not palpable. Serum bilirubin was 0.5 mg/100 ml.

The biochemical findings in this case are graphed (fig.64).

64



The initial very low values for the serum proteins should be noted, they may correlate with the ascites and ankle oedema. The hippuric acid and galactose tolerance tests returned to normal in the presence of the residual scarring in the liver.

The next case shows recovery from a much less severe zonal lesion.

Case 21. An 18-year old apprentice with infective hepatitis. Four days jaundiced, liver edge palpable 2 cm. below costal margin, spleen easily felt.

Serum bilirubin 2.7 mg/100 ml.

Liver sections show an essentially zonal lesion (fig.65). Portal tract infiltrations are very conspicuous and give the impression of chronicity.

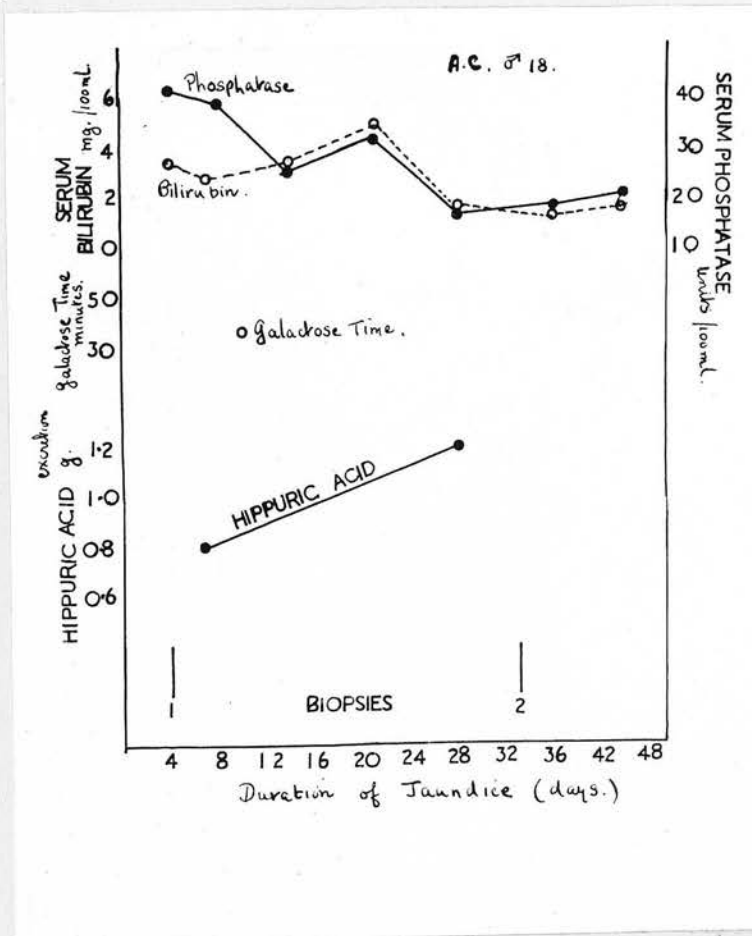
Although never deeply jaundiced the lad was slow to recover. Twenty nine days after the first biopsy serum bilirubin had reached 1.3 mg/100 ml.

A further aspiration biopsy was performed.

Biopsy 2. The lesion shows considerable regression (fig.66 and 67). The liver cell columns are restored right up to the central vein. Focal histiocytic accumulations are seen in the sinusoids. The portal tracts show residual fibrosis.

The biochemical findings are graphed (fig.68).

68.



The serum phosphates was the highest we have encountered in acute hepatitis. The reason for the

high phosphatase is not apparent from study of the histological picture. Even at the time of the first biopsy galactose tolerance and hippuric acid synthesis were normal.

The last case to be described was one in which zonal hepatitis was associated with fluctuant icterus of long duration. Recovery eventually occurred.

Case 22. A 30 year old soldier suffering from arsenotherapy jaundice, 8 days jaundiced, serum bilirubin 8.4 mg/100 ml.

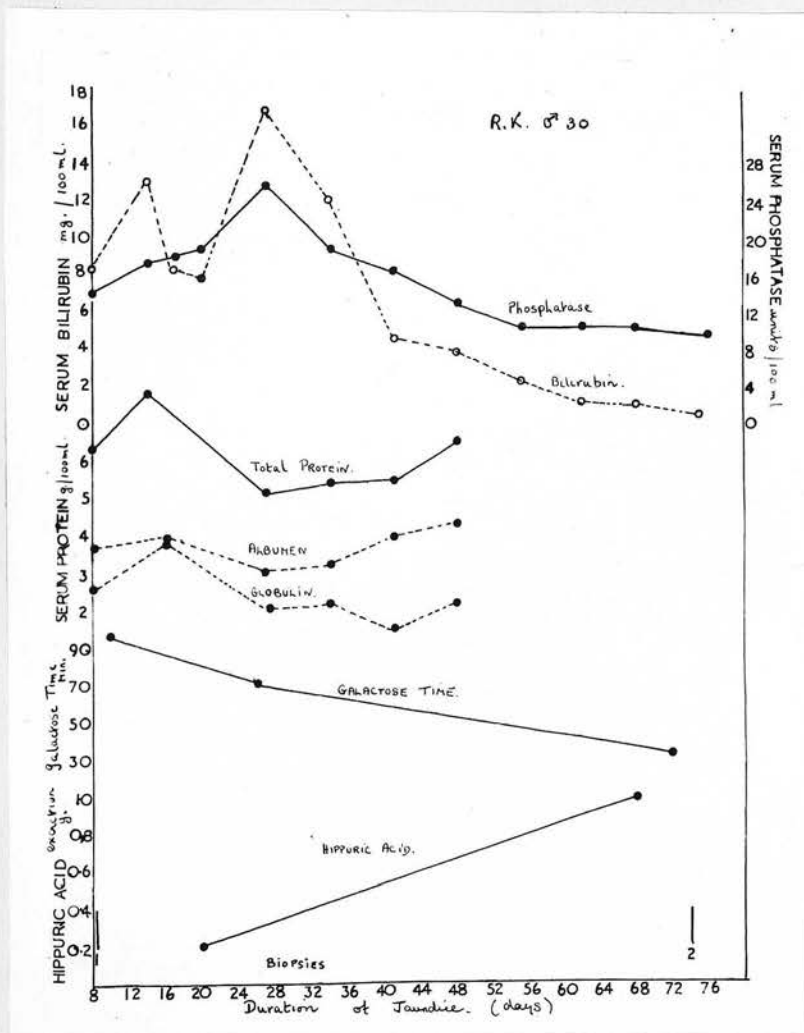
Sections show a fairly severe zonal hepatitis (fig.69). There is widespread infiltration of the sinusoids with round cells.

Jaundice waxed and waned and it was not until 9 weeks after the initial biopsy that the serum bilirubin fell to below 1 mg/100 ml. A further aspiration biopsy was performed.

Biopsy 2. (fig.70). Sections show complete recovery of liver structure. There is very slight residual portal fibrosis. K^upffer cells are prominent.

Biochemical results are graphed (fig. 71)

71.



The fluctuant course of the serum bilirubin has been mentioned. It should be noted that the galactose tolerance quickly returned to normal and was unaffected by subsequent exacerbations of **icterus**.

Hepatitis of zonal type seems to take the longest time to recover. There is a tendency for the icterus to relapse and the jaundice pursues a grumbling course, never entirely disappearing. This is shown in the last three cases described. However, despite the duration, the condition usually ends in

complete recovery. There is often residual portal tract fibrosis, but true cirrhosis is rare.

DISCUSSION.

It has been generally accepted that acute hepatitis, whether of epidemic or serum origin, is a disease from which recovery is usually complete (Roholm and Iversen, 1939, United States Army Circular Letter, 1942, Greene, 1943). There has been no increase in chronic hepatitis following the various war epidemics. Rössle (1930) for instance found a diminution in the incidence of cirrhosis after World War I. Lucké (1944b) made a study of the liver histology in 14 cases, dying from causes unrelated to the liver, who had suffered from acute hepatitis 1 week to 14 months previously. There was no evidence of permanent damage to the hepatic parenchyma, and restoration of the liver was practically complete. In our series sequelae have been rare and histological recovery was usually complete and rapid. The occasional exception in which the acute stage seemed to pass to permanent structural alteration in the liver will be observed in the next section.

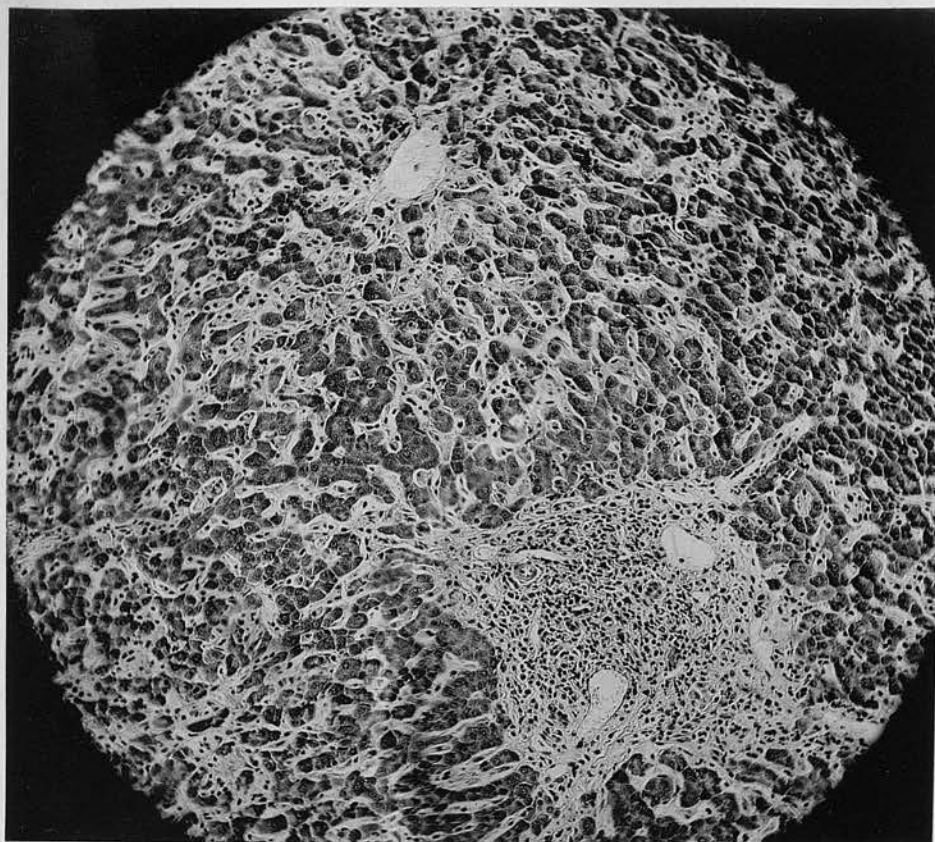
The basis for regular recovery of structure undoubtedly lies in the preservation of the reticulin framework. The liver damage is probably maximal at the outset, perhaps even in the preicteric stage (Barker, 1945). Continual cell damage with regeneration and fibrosis do not occur. Once the recovery process starts the regeneration of the hepatic parenchyma is amazingly rapid. The immense power

of regeneration of liver cells has been recognised in experimental animals (Whipple and Sperry, 1909, Fishback, 1929, Bollman & Mann, 1936). Our observations make it abundantly clear that this is true also of the human liver cell. With such speed of multiplication of liver cells it is surprising that mitoses are not more frequently observed. The centres of the lobules, the site of the poorest blood supply, are the last to reform. The periportal zonal fibrosis obviously creates some destruction to complete histological recovery and it is not surprising that scarring remains in that region after completion of recovery elsewhere. Moreover, it is the zonal type of lesion which most often leads to this residual change. Portal tract scarring after hepatitis has been mentioned by other authors, (Roholm and Iversen, 1939, Karsner, 1943, Lucké 1944b) its significance is a matter of some importance. Using modern terminology the lesion is not a cirrhosis, it does not disturb the lobular architecture, it is not associated with cell necrosis and regeneration. It does not seem to be progressive. The cases, the livers of which demonstrate it, do not present symptoms and the laboratory methods we have used have shown normal results. The scars therefore do not seem to prevent normal liver function. It is possible that the scars may ultimately resolve. The reversible fibrosis produced experimentally in animals by Cameron & Karunaratne (1936) may be recalled in this connection.

The cases described above have been studied for only a limited period after the acute attack.

In 7 additional cases the histology of the liver was studied by biopsy or autopsy material 6 months to 10 years after the initial attack of hepatitis.

A completely normal appearance was seen. Portal tract fibrosis was not evident. It is concluded that the normal course of acute hepatitis is to complete recovery, clinical, histological and biochemical.



65.

Case 21. Biopsy 1.

Zonal hepatitis. Portal tract infiltrations
are very conspicuous.

Best's carmine stain. X 112.

FIG. 66.

Case 21. Biopsy 2.

Recovery with residual portal fibrosis.

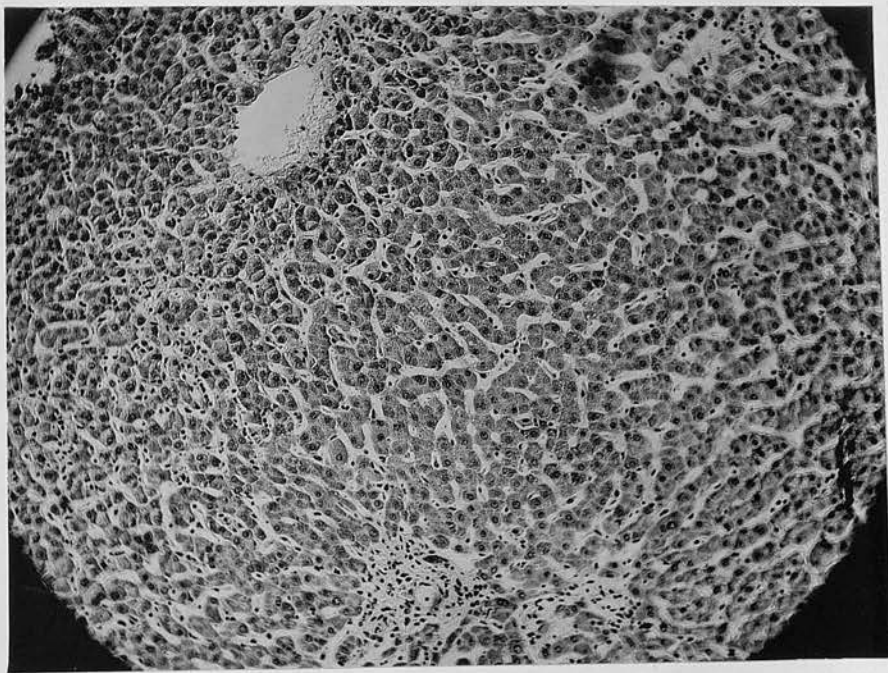
Best's carmine stain. X 115.

FIG. 67.

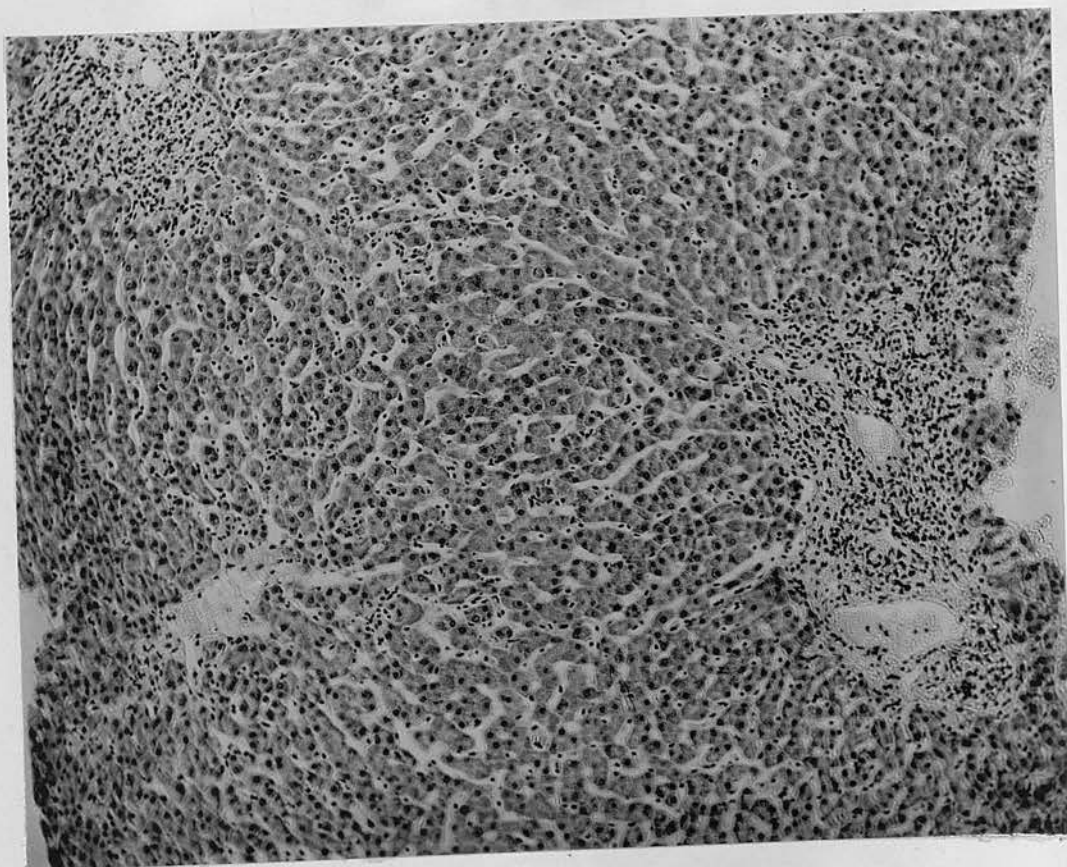
Case 21. Biopsy 2.

Another area showing recovery with minimal
portal scarring.

Best's carmine stain. X 112.



66.



67.

FIG. 69.

Case 22. Biopsy 1.

Zonal hepatitis. Inflammatory cells seen throughout the liver.

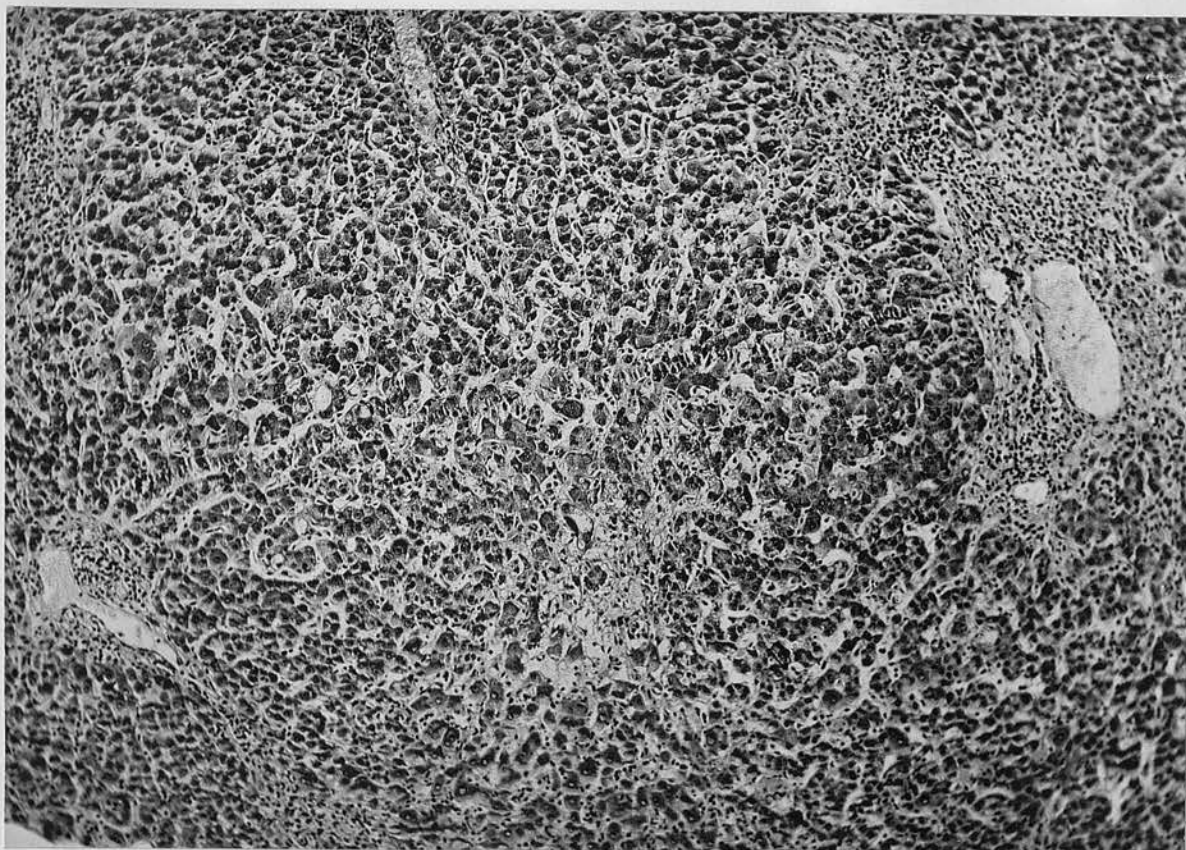
Best's carmine stain. X 105.

FIG. 70.

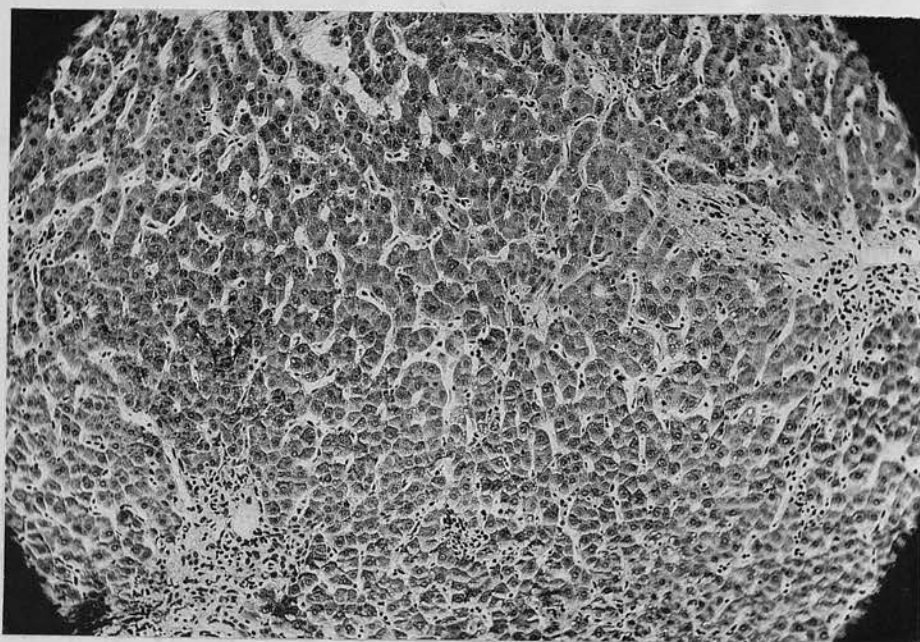
Case 22. Biopsy 2.

Recovery. Very slight residual portal scarring.

Best's carmine stain. X 112.



69.



70.

FIG. 72.

Case 23. Biopsy 1.

Severe mixed type of hepatitis. Central autolysis of liver cells with dissociation of remaining liver cells which show numerous mitoses. Heavy round cell infiltration of portal tract.

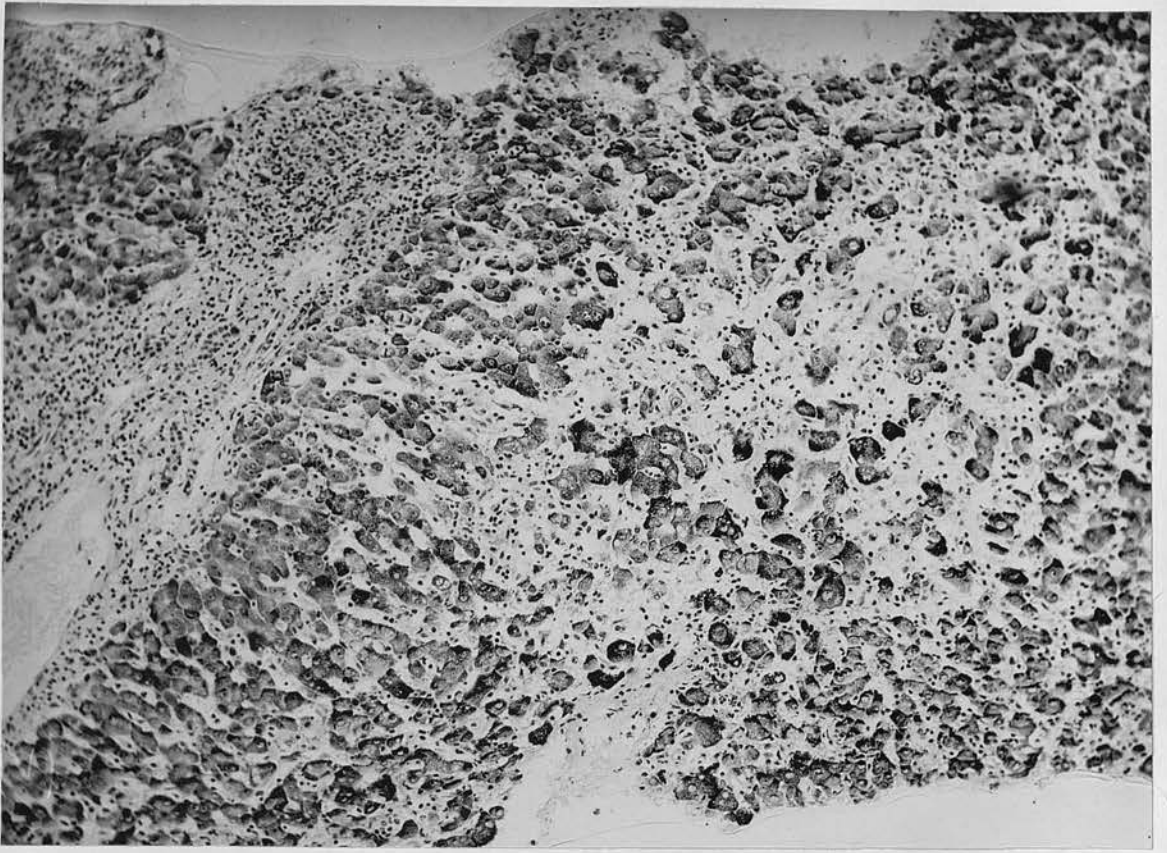
Best's carmine stain. X 115.

FIG. 73.

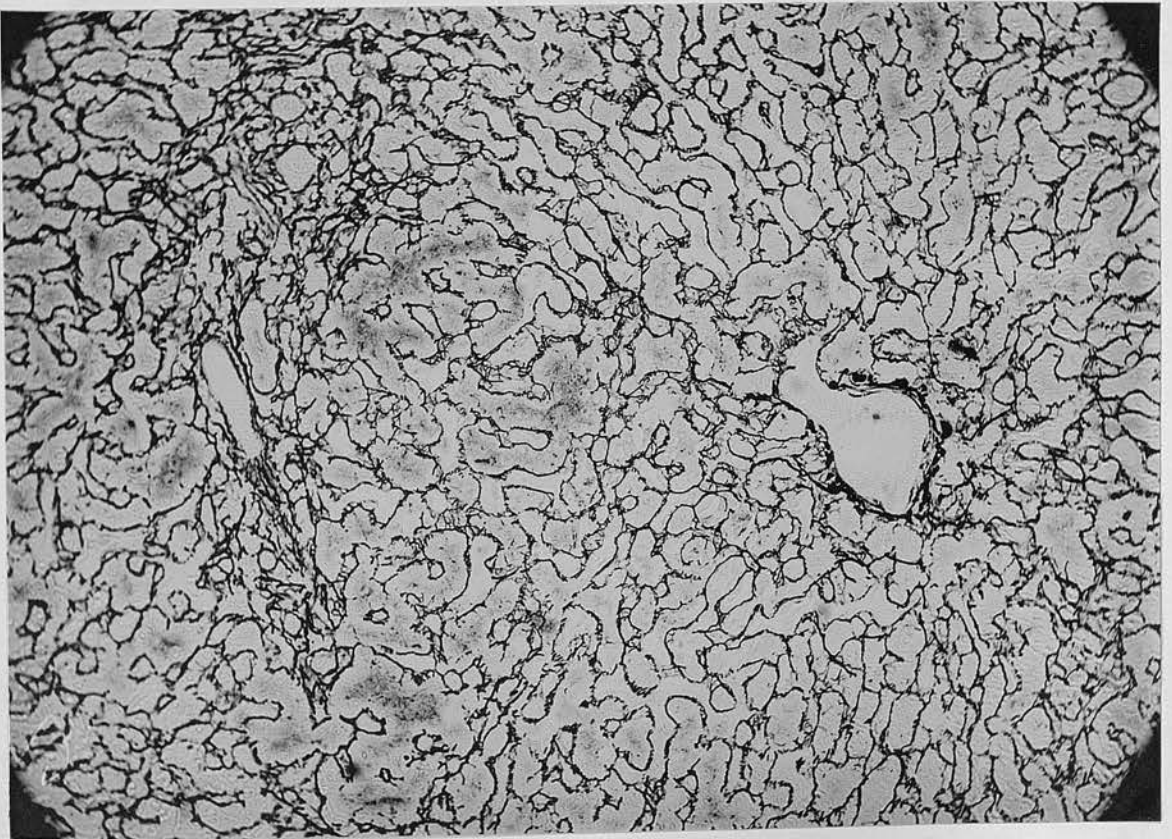
Case 23. Biopsy 1.

Some reticulin increase in the portal tract but good preservation of the reticulin pattern.

Modified Foot's stain. X 160.



72.



73.

FIG. 74.

Case 23. Biopsy 2.

Persistence of severe hepatitis.

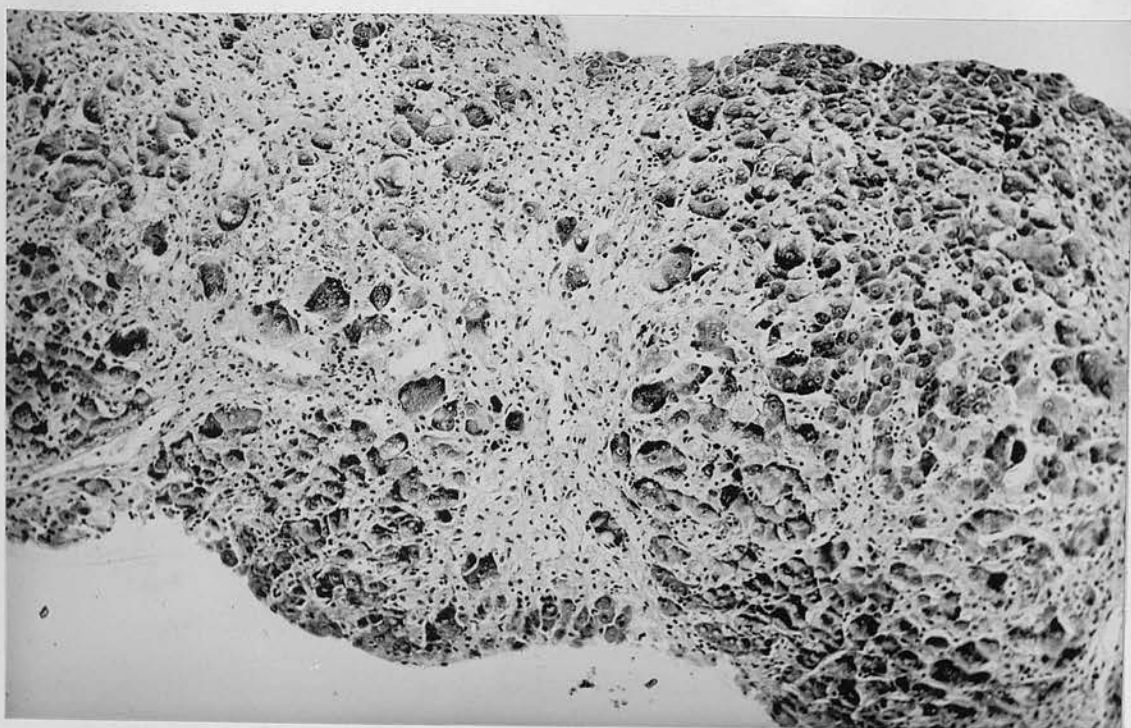
Best's carmine stain. X 115.

FIG. 75.

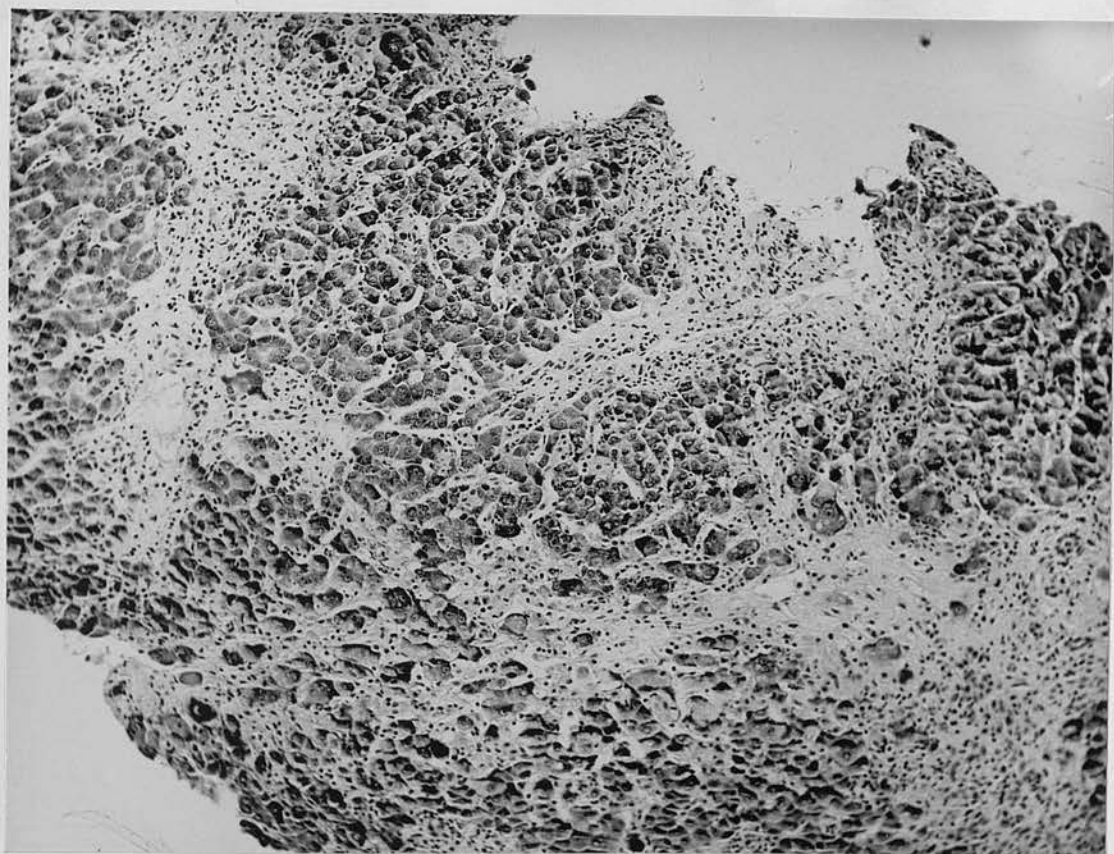
Case 23. Biopsy 2.

Another area showing tendency to separation
of nodules of liver tissue by fibrous bands.

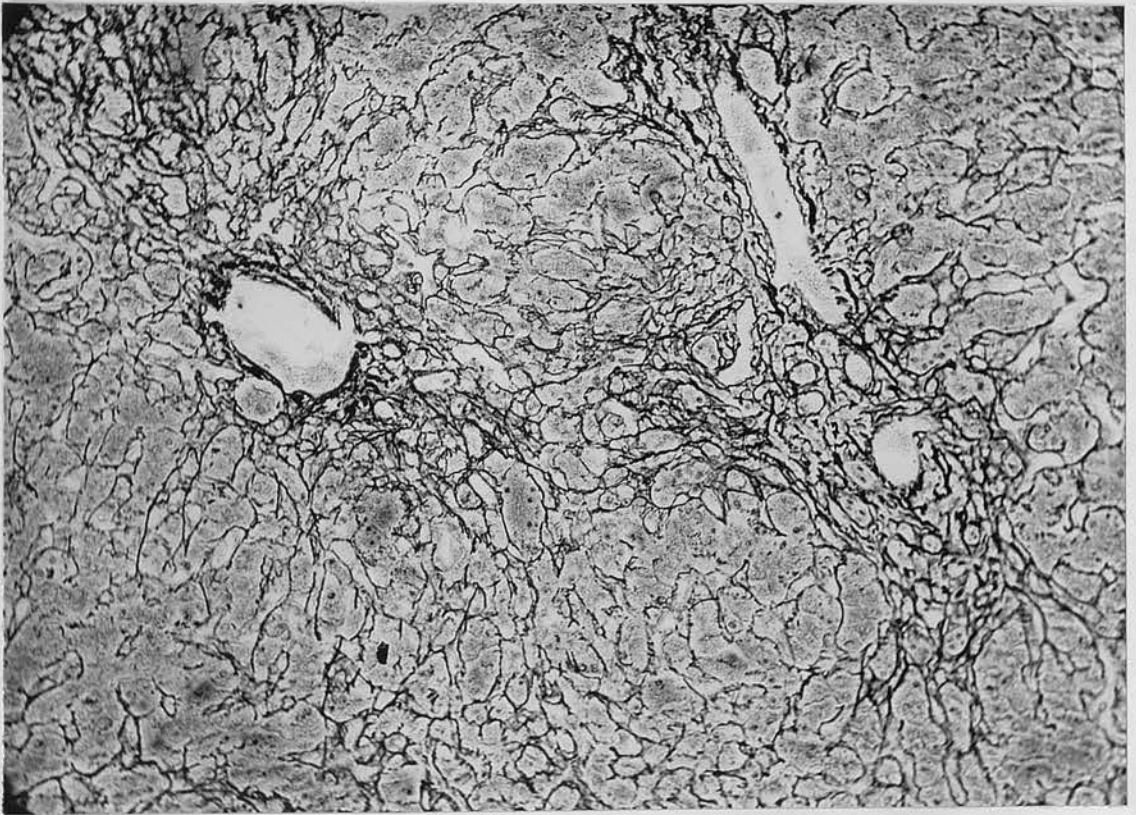
Best's carmine stain. X 115.



74.



75.



76.

Case 23. Biopsy 2.

Reticulin increase in portal tracts and
around central vein. Fusion of two into
a fibrous band.

Modified Foot's stain. X 160.

FIG. 77.

Case 23. Biopsy 3.

Fully developed cirrhosis.

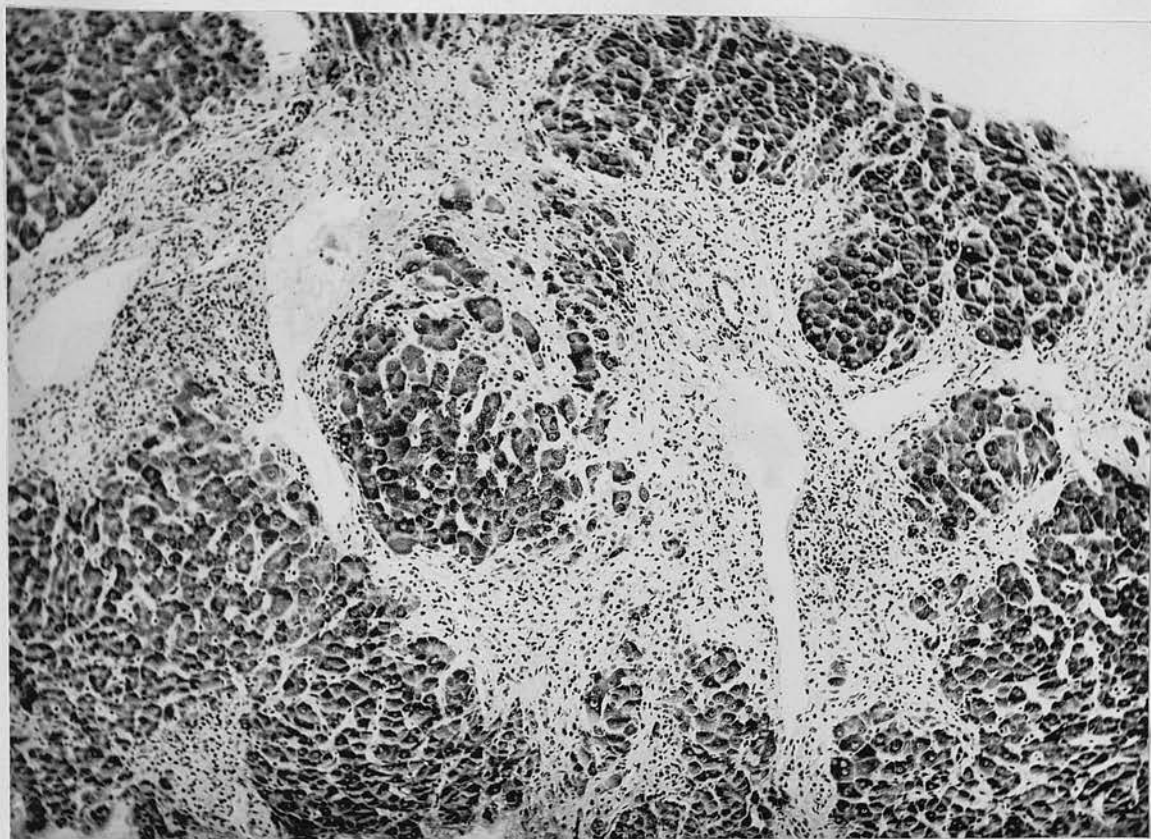
Best's carmine stain. X 115.

FIG. 78.

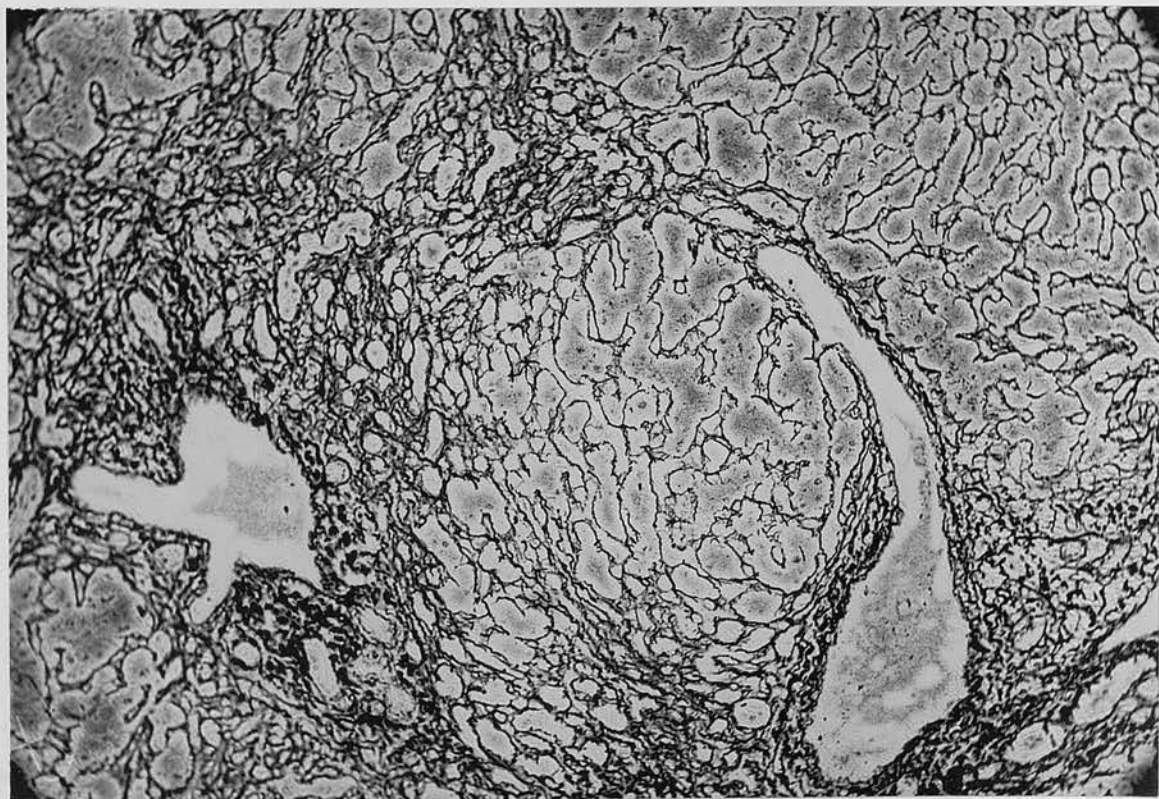
Case 23. Biopsy 3.

Reticulin stain showing isolation of nodules
of liver tissue by bands of reticulin fibres.

Modified Foot's stain. X 160.



77.



78.

FIG. 80.

Case 24. Biopsy 1.

Classical cirrhosis with nodular area of liver cells and new bile duct formation in the fibrous tissue.

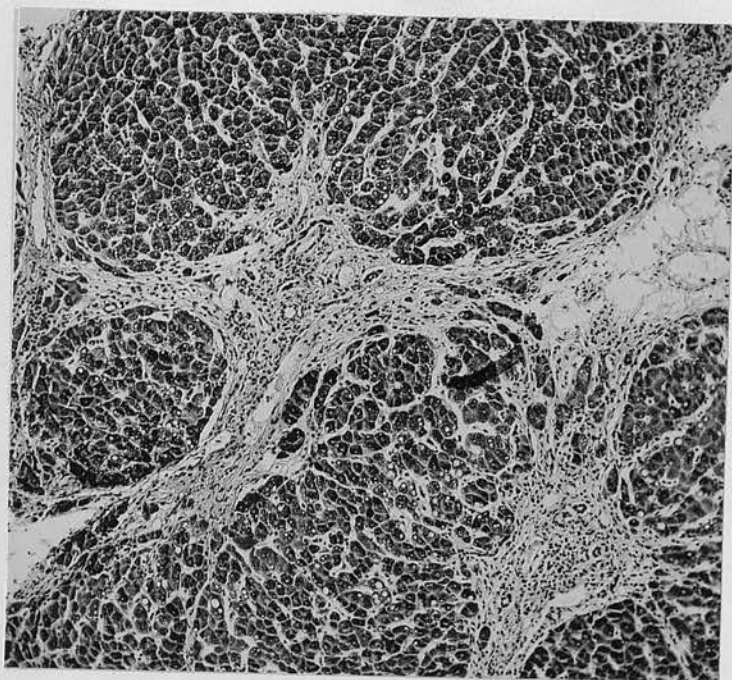
Best's carmine stain. X 80.

FIG. 81.

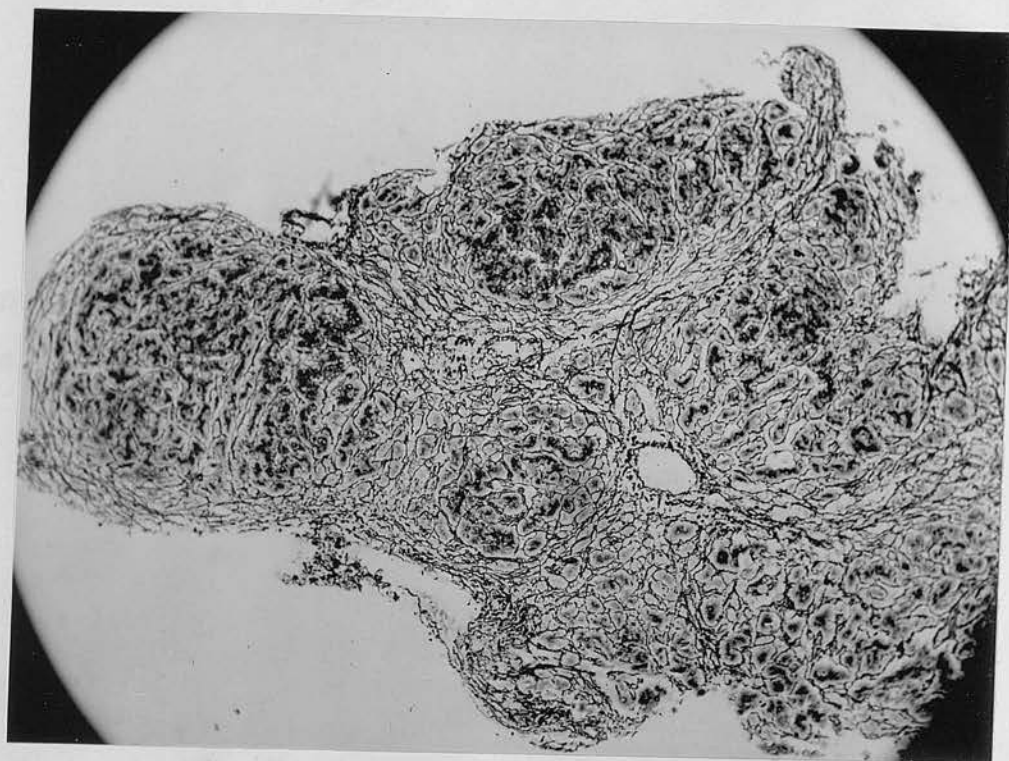
Case 24. Biopsy 1.

Reticulin bands isolating nodules of liver tissue.

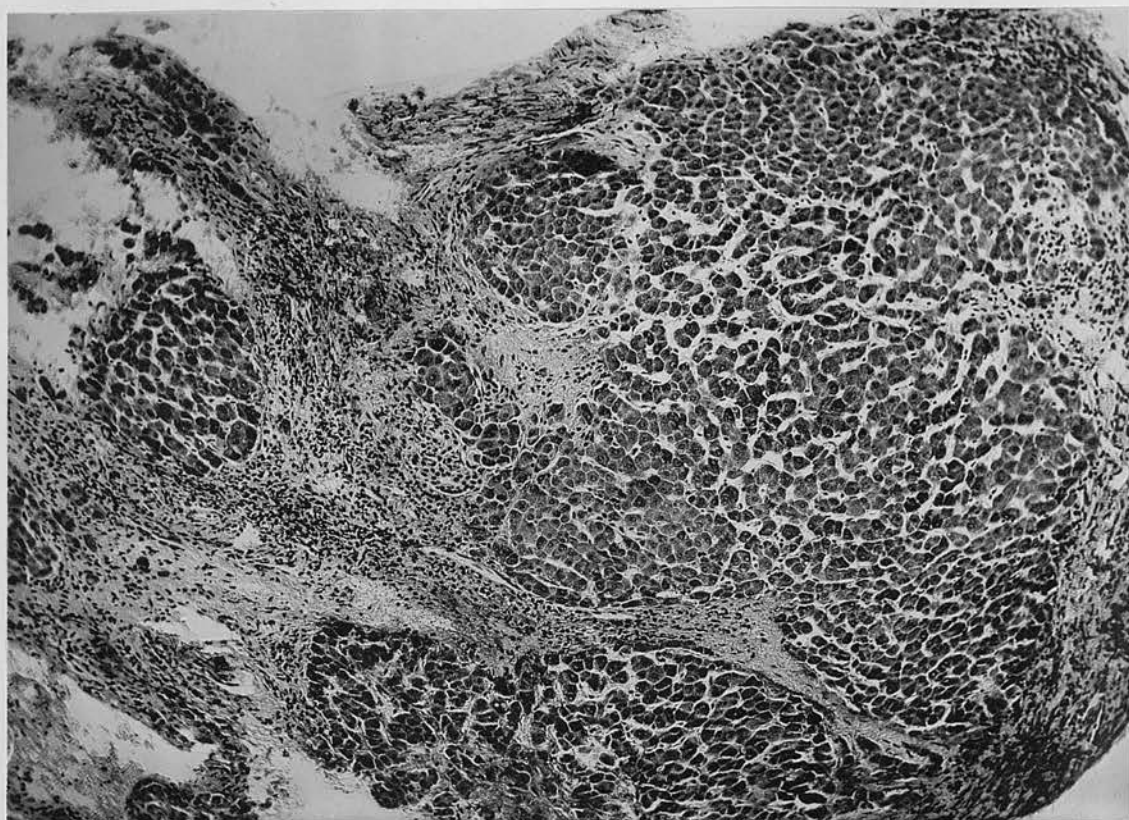
Modified Foot's stain. X



80.



81.



82.

Case 24. Biopsy 2.

Inactive cirrhosis. No acute change seen.

Best's carmine stain. X 105.

CHAPTER 13.

POST-HEPATITIS CIRRHOSIS.

Although an attack of acute hepatitis usually passes to complete cure, from time to time long term sequelae have been reported in the literature. Jones & Minot (1923) describe 2 cases of cirrhosis, clinically diagnosed, occurring in young persons after "catarrhal jaundice". Polack (1937) mentions 8 cases in which chronic hepatitis developed after acute epidemic hepatitis. Other authors, using the bilirubin excretion test, have found permanent impairment of liver function many years after an attack of acute hepatitis (Soffer and Paulson, 1934; Kornberg, 1942). Altschule and Gilligan (1944) investigated a series of 36 unselected subjects 1-29 years after an attack of acute hepatitis. 25 per cent showed excess bilirubin in the blood, some had slight hepatomegaly, there were no symptoms referable to the liver. In most of the reported cases hepatic histology was not available and the nature of the underlying liver lesion remained in doubt. This doubt was largely cleared by the work of Krarup & Roholm (1941). These authors, using the aspiration biopsy technique, examined 12 patients with a severe protracted or recurring hepatitis. They found a gradual transition from the usual acute hepatitis picture to that of a fully developed Laennec's cirrhosis. Our series consists of 6 cases of which a

histologically confirmed hepatic cirrhosis could reasonably be related to a preceding attack of acute hepatitis. These cases will be described and the laboratory findings discussed.

Methods. The methods used for the estimation of serum bilirubin, serum phosphatase, serum cholesterol and serum plasma proteins and for the performance of the intravenous galactose tolerance test and the hippuric acid synthesis test have been described previously.

Urobilinuria. Excess of urobilin in the urine was tested for by Schlesinger's Alcoholic zinc acetate method.

Bromsulphthalein Excretion Test. The method employed was that of Helm and Machella (1942). 5 mg. per kilo body weight of the dye in 5 percent solution is injected intravenously. Samples of venous blood are taken 5 and 30 minutes after injection. A retention of more than 10 per cent of the standard is considered abnormal.

Colloidal Gold Reaction. In some cases the colloidal gold reaction was performed on the serum according to the technique of MacLagan (1944).

Case Descriptions.

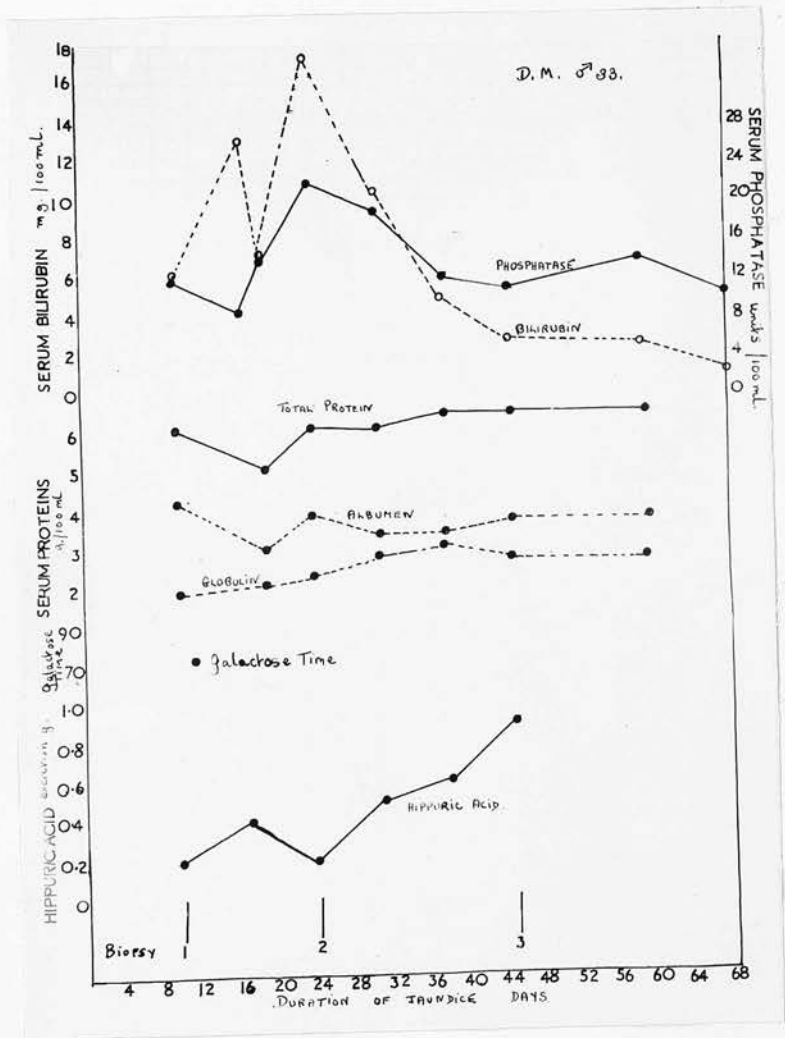
The first case is one in which the initial acute lesion was followed through to cirrhosis.

Case 23. A 33 year old man suffering from arsenotherapy jaundice. 10 days jaundice, liver edge palpable 6 cm. below right costal margin, spleen just tipped. Serum bilirubin 6.2 mg. per 100 ml.

Liver biopsy sections show a fairly severe mixed hepatitis. (Fig. 72). The portal tract infiltrations are very cellular. The centres of the lobules show severe autolytic change. Surviving liver cells stand out among the areas of necrosis. These cells show numerous mitoses. There is some increase of reticulin in the portal tracts but on the whole the reticulin framework is well preserved (fig. 73).

The very moderate icterus, the presence of urobilin in the urine and the histology of the liver anticipated a rapid recovery. However the patient became more jaundiced and 14 days after the first biopsy the serum bilirubin was 17.5 mg. per 100 ml. Urobilin had disappeared from the urine. A second liver biopsy was undertaken.

The second biopsy shows a very similar picture to that seen originally, (fig. 74). Again there is very severe cell necrosis especially at the centres of the lobules. The autolysing liver cells have lost their complement of glycogen. This appearance however was not constant throughout the section. Other areas showed a different picture (fig. 75.). Here nodules of liver tissue seem to have been isolated by lines of necrosis and fibrosis. Moreover reticulin stains of these areas demonstrate conspicuous reticulin condensation in the central areas and in the portal tracts and there is a tendency for the two to become linked by bands of fibrous tissue (fig. 76).



These findings suggested that a hepatic cirrhosis might develop and although further clinical recovery proceeded normal, it was considered important to perform a third biopsy. This was undertaken 3 weeks after the second, on the 45th day of jaundice. The serum bilirubin was 2.8 mg. per 100 ml.

Sections of the third biopsy show a definite cirrhosis (fig. 77). Bands of connective tissue containing numerous round cells and proliferating bile-ducts disturb the normal lobular architecture. Nodules of hepatic cells showing regeneration have been isolated. Cell autolysis is apparently proceeding at the periphery of the nodules. A large number of normal looking liver cells remain. Reticulin stains confirm the development of cirrhosis (fig. 78).

The laboratory findings are graphed (fig. 79) and included in table 29. The patient was discharged from hospital symptom free. Liver and spleen are not palpable. The laboratory methods employed give normal results. On discharge the serum bilirubin was slightly increased, 3 months later it is normal. The cirrhosis is in a latent phase. Its development would not have been recognised if serial liver biopsies had not been performed. The prognosis of a patient with such a hepatic lesion is uncertain. This case has not been studied for long enough for a dogmatic statement to be formulated. The case is described to demonstrate the mode of development of

cirrhosis from acute hepatitis and to indicate that such a lesion can occur after hepatitis which runs an apparently normal course, and without residual untoward signs and symptoms. There are no readily detectable biochemical abnormalities.

The next case is one which has been studied over a longer period. At no time has activity of the cirrhosis been demonstrated.

Case 24. In May 1942 a 29 year old soldier suffered from arsenotherapy jaundice. Clinically the attack was a severe one. He was deeply jaundiced. Ascites occurred and required paracenteses on four occasions. Clubbing of fingers developed. Jaundice cleared in two months. Five months later, September 1942, he was referred to Hammersmith Hospital for investigation. He was symptom free. The liver edge was palpable 2 cm. below the right costal margin. The spleen could just be tipped. Serum bilirubin was 0.2 mg. per 100 ml.

Liver sections showed a classical cirrhosis with nodular areas of liver cells and new bile-duct formation in the fibrous tissue (figs. 80 and 81).

In June 1944 the patient volunteered for a further biopsy. In the intervening 21 months he had remained well. Digestion was excellent and he was satisfactorily carrying out his duties as telephone operator in the Army. Liver and spleen were not palpable.

Serum bilirubin was less than 0.5 mg. per 100 ml.

Biopsy 2.- Sections still show a very definite cirrhosis. There is little change from the biopsy of 21 months ago. All evidence of acute change has disappeared. There seem to be plenty of normal looking liver cells (fig. 82).

In June 1945 the patient reported. He had remained perfectly well. Liver and spleen are not palpable. Serum bilirubin is less than 0.5 mg. per 100 ml.

This patient has had a known cirrhosis for three years. During this time he has been symptom free. The future outlook is still uncertain.

The next case is of an elderly woman in whom cirrhosis developed after epidemic hepatitis. The patient has been under observation for the subsequent $2\frac{1}{2}$ years.

Case 25. A 71 year old woman suffered from epidemic hepatitis. There was a fluctuant jaundice for 5 months. At this time the liver was enlarged to the umbilicus and the serum bilirubin was 4.4 mg. per 100 ml.

Sections show a very chronic lesion with liver cells split into groups by the fibrous tissue, early nodular hyperplasia and bile duct proliferation in the fibrous portal tracts. (Fig. 83).

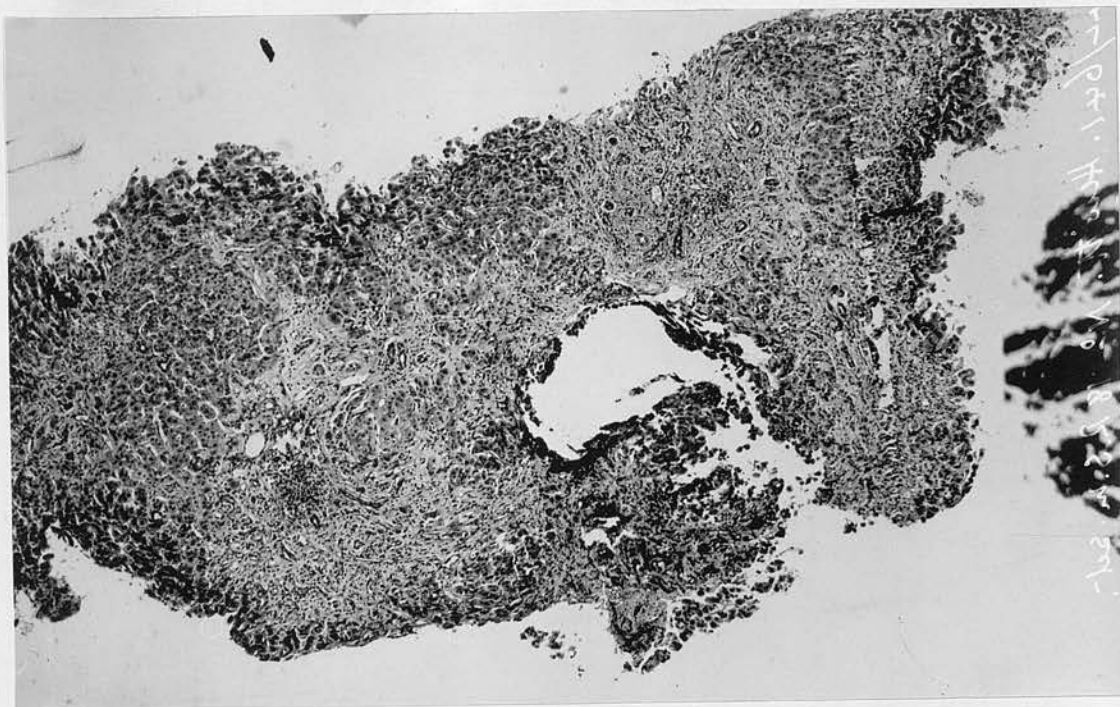
The jaundice subsided during the next 7 weeks and

the patient was discharged from hospital symptom free. A year later she reported for a routine follow-up examination. She had gained a stone in weight. The liver edge could now be felt only 2 cm. below the right costal margin. She volunteered for a further aspiration biopsy. An adequate sample was obtained with some difficulty. When pierced with the trocar the liver tissue felt very hard and gritty and the biopsy was very fragmented. Sections showed little clumps of liver cells surrounded by wide bands of fibrous tissue containing proliferating bile ducts. All evidence of cell necrosis have disappeared.

The patient was next admitted 18 months later with a mild gastro-intestinal upset. There had been epigastric pain, nausea and vomiting for 2 days. She recovered in a week. Apart from this attack she had remained well and there had been a further gain in weight. Routine biochemistry was done but it was not considered necessary to perform a third biopsy.

The 3 cases (23, 24 & 25) described above are examples of cirrhosis following hepatitis in which the liver lesion is neither showing histological evidences of activity nor is associated with symptoms. The next 3 cases are of the same condition but in which the liver shows varying degrees of activity.

Case 26. A 41 year old Canadian Soldier. In June 1944, while in Italy, he complained of anorexia,



83.

Case 25.

A very chronic lesion with liver cells split up into groups by bands of fibrous tissue containing proliferating bile ducts.

H.E. X 70.

FIG. 84.

Case 26.

Cirrhosis. Very active lesion. Liver tissue replaced by areas of fibrous tissue heavily infiltrated with round cells and containing an occasional bile duct or group of necrotic liver cells.

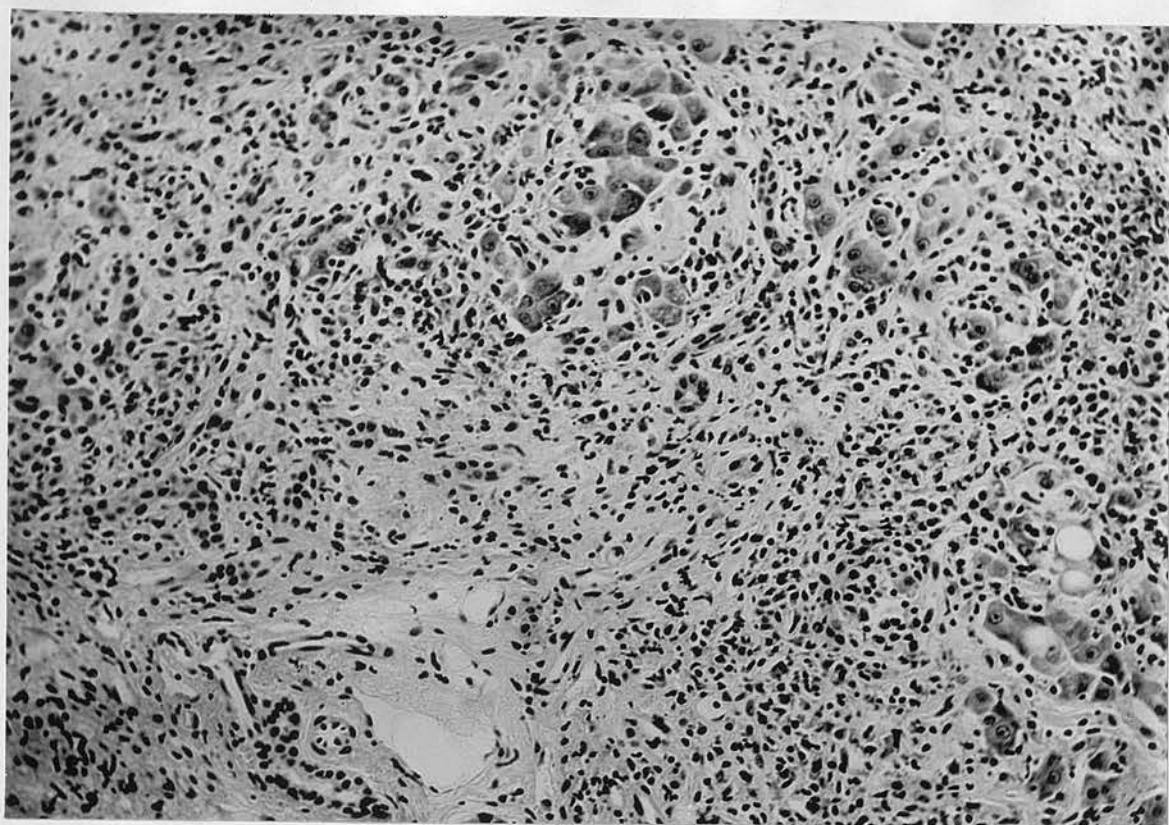
Best's carmine stain. X 220

FIG. 85.

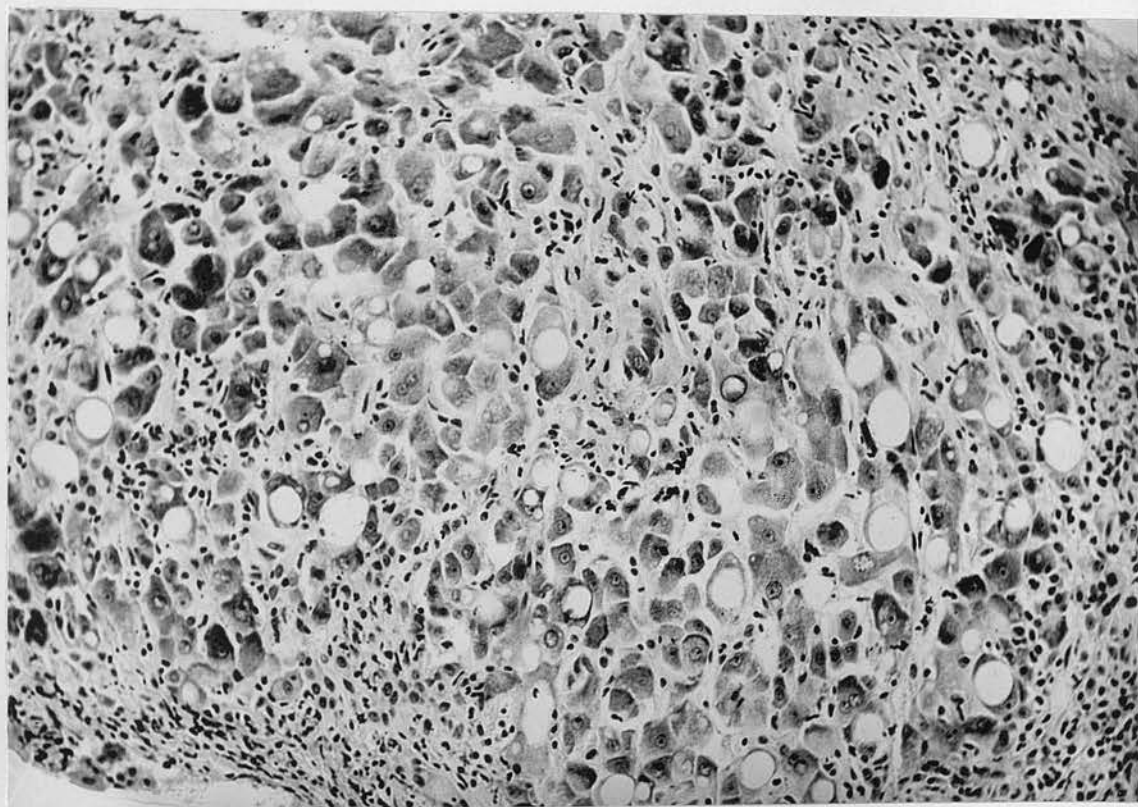
Case 26.

A further field. Remaining liver cells show degenerative changes. Some are infiltrated with fat; others contain vacuoles or show excessive granularity.

Best's carmine stain. X 220.



84.

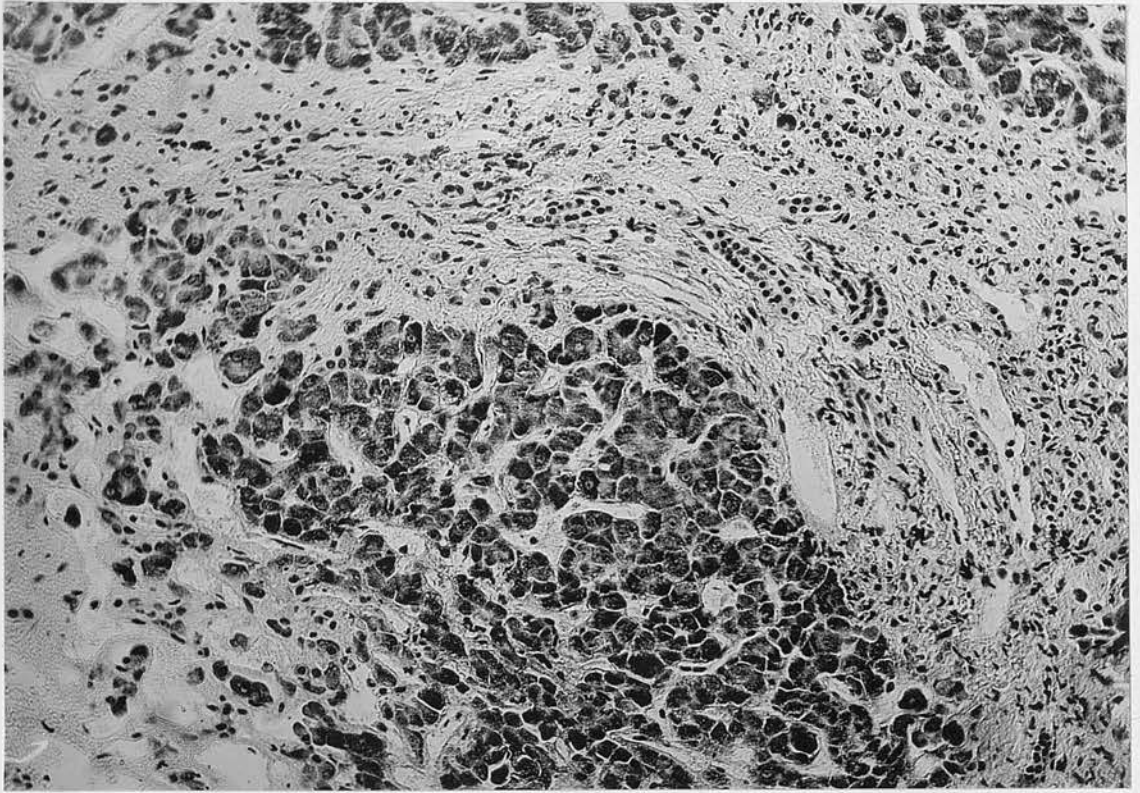


85.

vomiting and diarrhoea. There was no clinical jaundice but at that time infective hepatitis was epidemic in his unit. Treated by the regimental medical officer. In September 1944 the patient was hospitalised because of a further bout of vomiting and diarrhoea. The liver edge was found 6 cm. below the right costal margin. During the succeeding 6 months there were numerous hospital admissions with similar complaints. He became very depressed and lost 30 lbs. weight. Amoebae were never seen in the stools. Sigmoidoscopy was normal. In March 1945 he improved, gained weight and all symptoms disappeared. The liver remained enlarged and in May 1945 he was referred to Hammersmith Hospital for investigation. The liver was palpable 6 cm. below the right costal margin, the spleen could just be tipped. The serum bilirubin was 2.2 mg/100 ml.

Sections show a severe active cirrhosis. Some fields show only mixed round cells and fibroblasts with an occasional bile duct (fig.84). A few isolated liver cells show degenerative changes. Other areas, (fig.85) show groups of highly abnormal liver cells. Many of the cells are distended with fat globules. Others show excessive granularity and vacuolation of their cytoplasm. Some have lost their nuclei.

The most likely aetiological factor in the production of this active cirrhosis is a mildly icteric



86.

Case 27.

Post hepatitis cirrhosis. Remaining liver cells look fairly normal. The fibrous bands are relatively acellular and contain proliferating bile ducts.

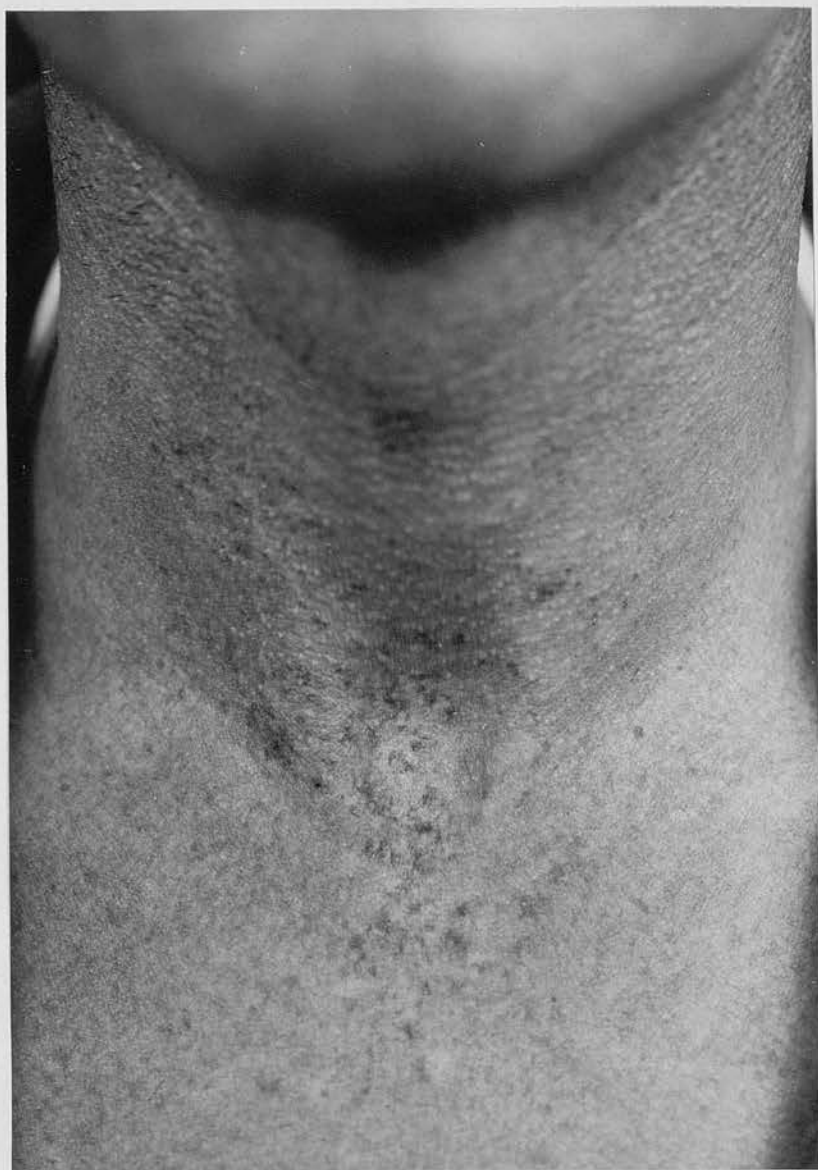
Stained Best's Carmine. X 190.

infective hepatitis. The disease was epidemic at the time the hepatomegaly was first discovered. With such an active lesion it is surprising that the patient is free of symptoms.

The next case is also of cirrhosis following infective hepatitis. The liver lesion is less active than that described in case 26.

Case 27. In March 1942 a 22 year old soldier contracted epidemic hepatitis. The acute attack was not unduly severe. He was jaundiced for 3 weeks and convalescent for a further 7 weeks. Since then he has not felt perfectly fit, and on exerting himself has complained of nausea and right abdominal discomfort. His colour changes to a greenish hue "like a bilious attack". In November 1943 he was referred to Hammersmith Hospital for investigation. He was a well developed man. The liver was palpable 4 cm. below the right costal margin. The spleen was not felt. The serum bilirubin was 1.5 mg/100 ml.

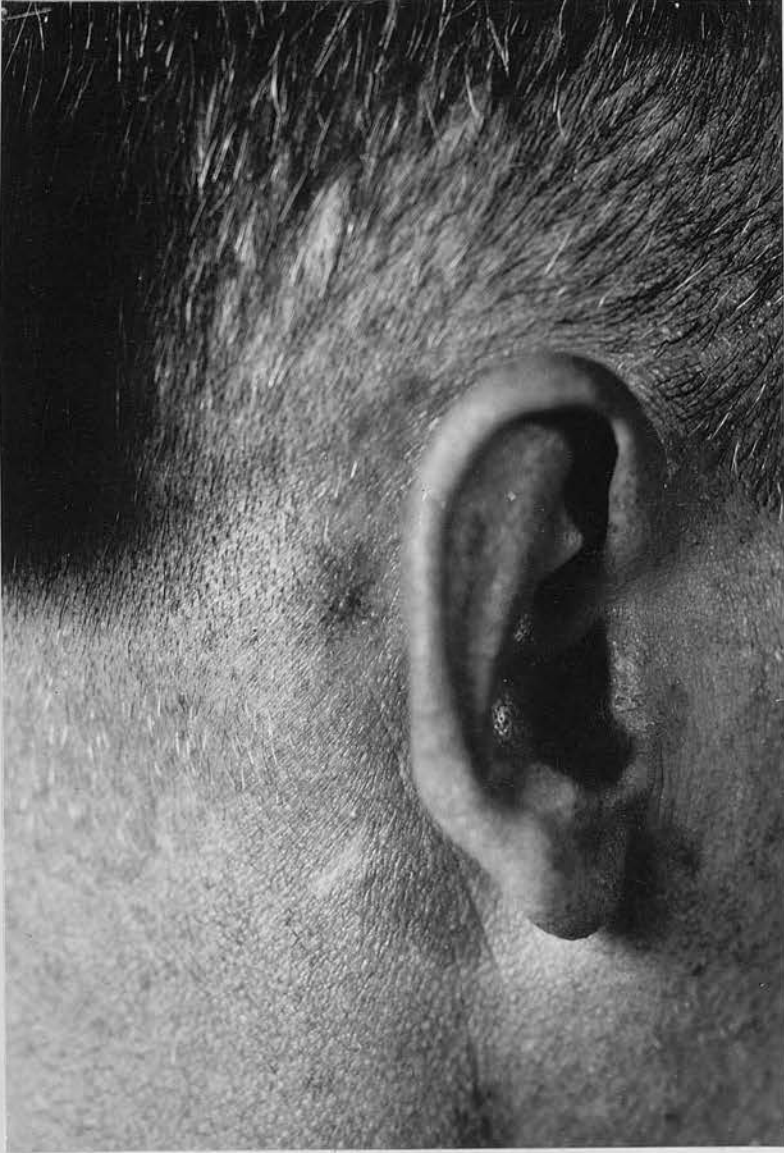
Sections show a definite cirrhosis (fig.86). The lesion does not appear so active as that depicted in case 25. The surviving liver cells appear very normal. The bands of fibrous tissue are relatively acellular. Bile duct proliferation is conspicuous. The use of an exercise test to determine the fitness of patients recovering from hepatitis has been advocated by Benjamin & Hoyt (1945). Barker (1945)



87.

Case 28.

Spider naevi over necklace area.



88.

Case 28.

Large spider naevus behind right ear.

The centre of this telangiectasis was raised,
pressure on it with a pin-head causing
blanching of the spider.

recommends a "jeep" test. A rough riding experience to make sure that the disease process in the liver had died out. Case 27 was given an exercise test. After 15 minutes brisk walking on the flat followed by 2 flights of stairs he complained of nausea, he looked a sallow yellow colour and the right upper abdomen was tender and swollen. There had been no significant change in the serum bilirubin level.

The last case in this group is of cirrhosis following arsenotherapy jaundice. When first seen the disease appeared fairly active. It now seems quiescent.

Case 28. In February 1944 a 33 year old soldier suffered from arsenotherapy jaundice. He was slow to recover, the jaundice continuing for 3 - 4 months. In July 1944 he became very jaundiced and was re-admitted to another hospital. His serum bilirubin was said to be 20-20 mg./100 ml. He made a slow recovery and in January 1945 he was discharged from the Army. On 4th April 1945 he was referred to Hammersmith Hospital. He complained of general malaise, dyspnoea and discomfort in the left side on exertion. Occasionally he passed a pale stool and the urine was darker than usual. Frequent epistaxis occurred on blowing the nose. Appetite and digestion generally were excellent. The patient was a thin sallow man. Spider naevi of the type described by Bean (1943) in chronic liver disease

FIG. 89.

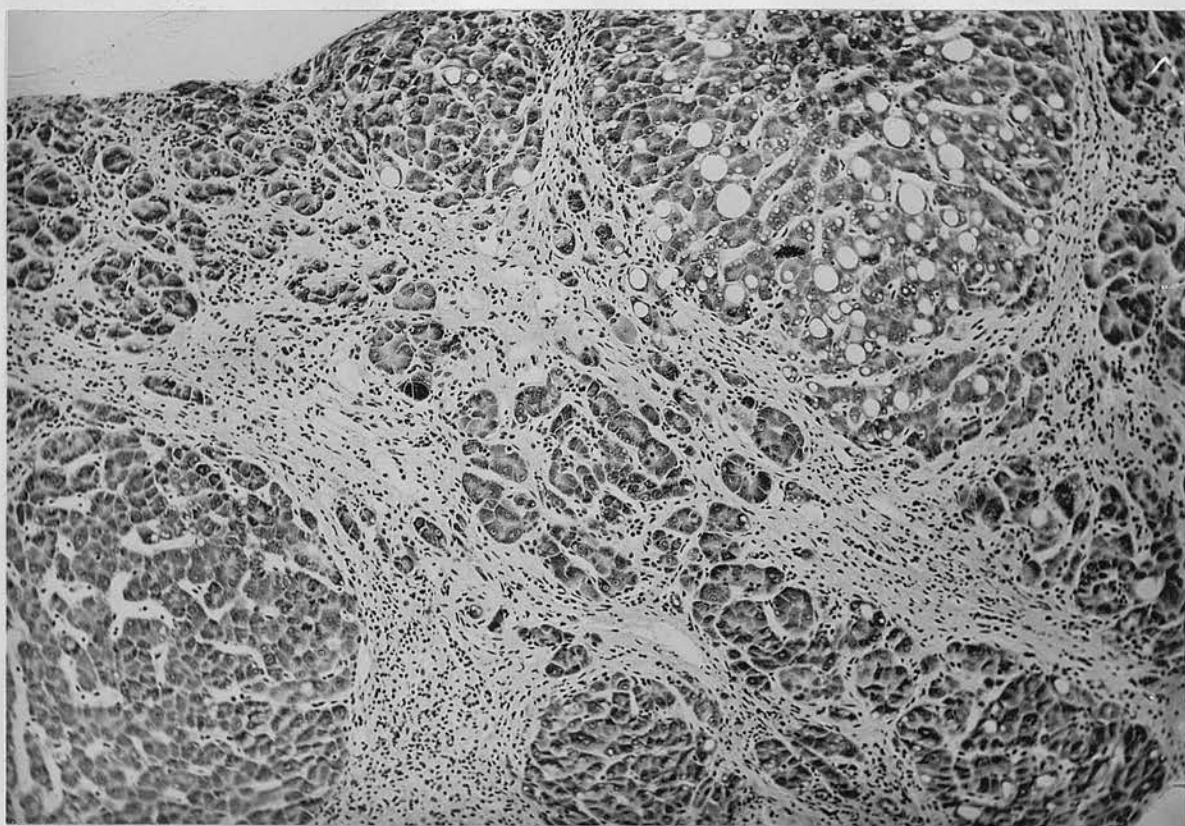
Case 28. Biopsy 1.

Post-hepatitis cirrhosis. Fatty change
in liver cells at periphery of nodule.
Best's carmine stain. X 115.

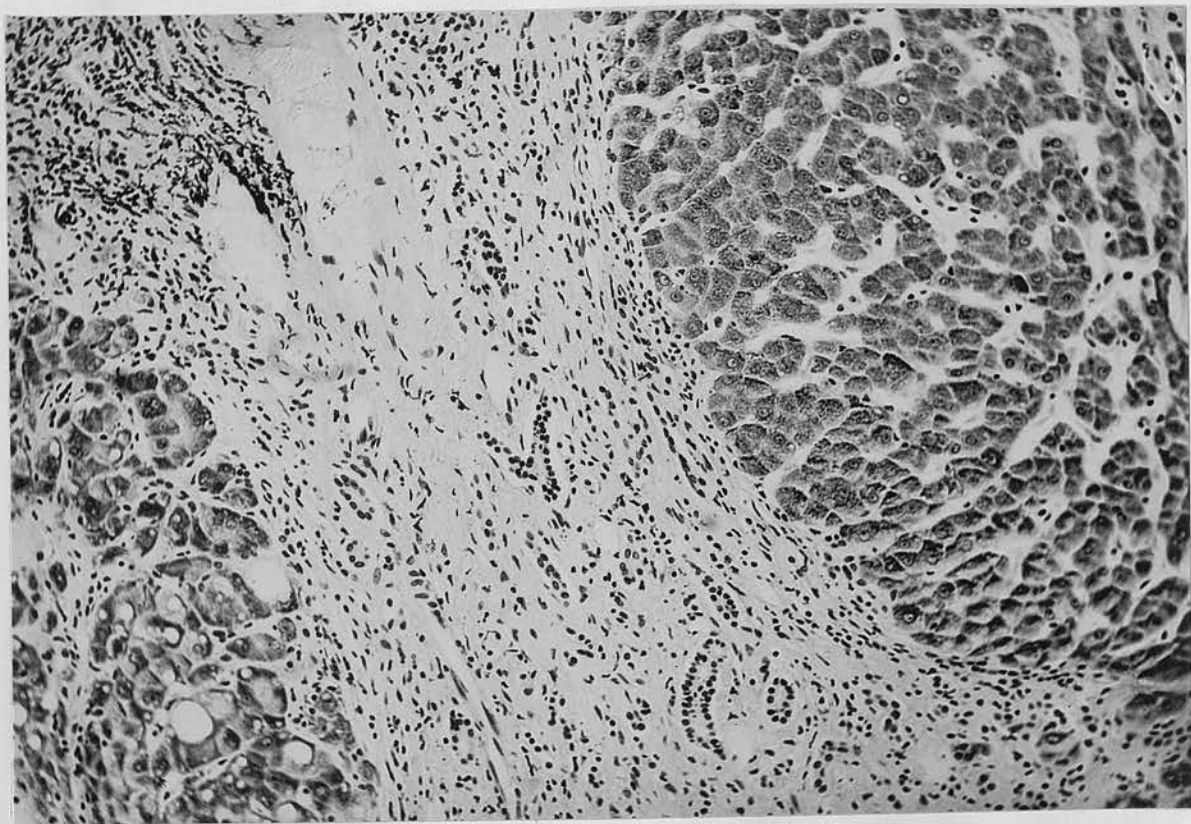
FIG. 90.

Case 28. Biopsy 1.

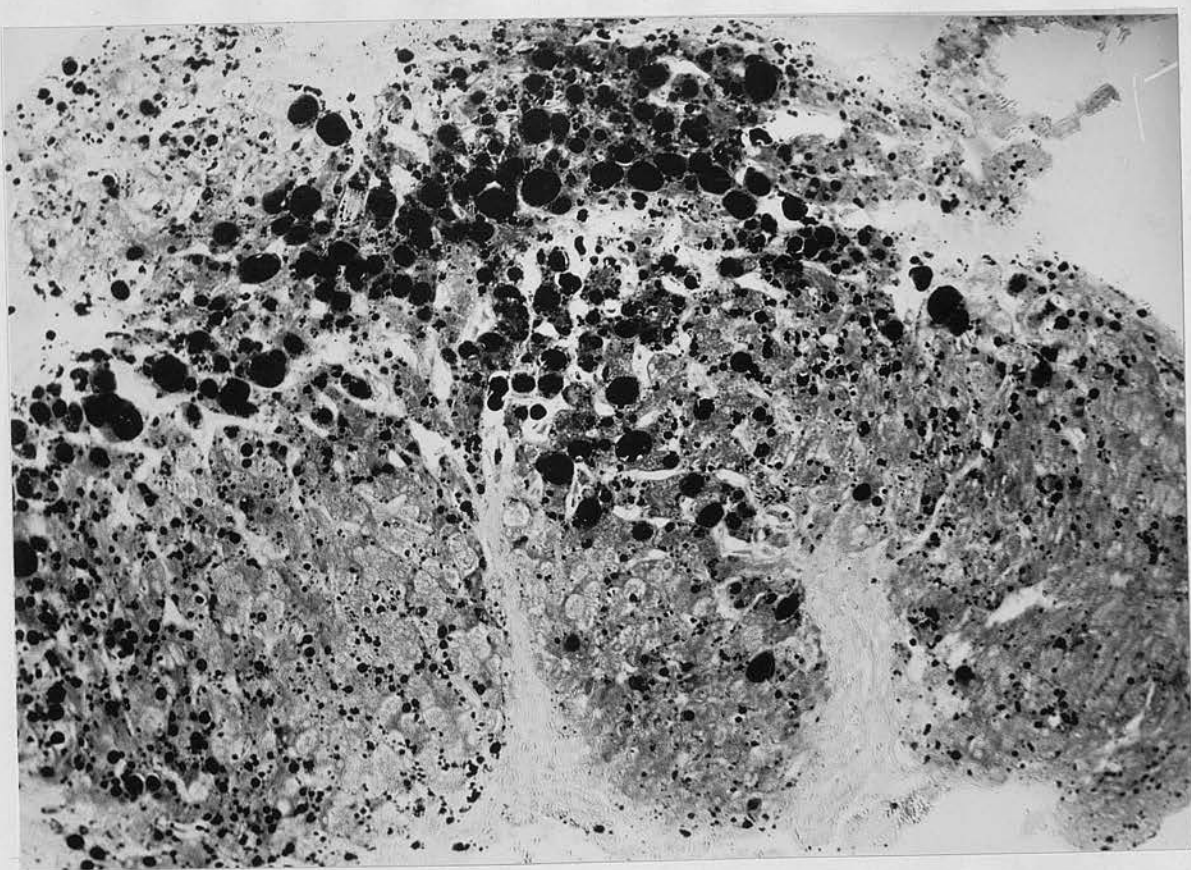
Magnified view of fibrous band. Band
is relatively acellular and contains
numerous bile ducts. Adjoining liver
cells apart from fatty change appear
fairly normal.
Best's carmine stain. X 175.



89.



90.



91.

Case 28.

Fatty change in liver at periphery of
lobule.

Osmic acid stain. X 130.

were seen on the forehead, necklace area and wrists (figs.87 and 88). The spleen was enlarged half way to the umbilicus. The liver margin was tender and just palpable below the right costal margin. Serum bilirubin was 0.7 mg./100 ml.

Liver sections show a cirrhosis (fig.89). Fibrous bands have cut off nodules of liver tissue. The fibrous bands are relatively acellular and contain numerous bile ducts (fig.90). The liver cells towards the periphery of the nodules show fatty change (fig.91).

A further biopsy was performed 5 weeks after the first. There is no significant change in the appearance of the sections.

This patient reported 3 months later. He is now symptom free. He is back at work. The vascular spiders are less conspicuous. The spleen now extends only 4 cm. below the left costal margin. Laboratory findings are now normal. It may be that the cirrhosis has now passed into an inactive state. The treatment of this case will be discussed in some detail later.

Laboratory findings in post hepatitis cirrhosis.

The laboratory data in these 6 cases are tabulated (Table 29). It is obvious that such a case as No. 25 which presents bilirubinaemia, hyperglobulinaemia reversal of the A/G ratio and abnormality of the colloidal gold reaction, the bromsulphthalein,

TABLE 29

THE LABORATORY FINDINGS IN 6 CASES OF POST HEPATITIS CIRRHOSIS.

Case	Age & Sex	Type of hepatitis	months after hepatitis	Date	Liver	Spleen	Urobilin-uria	Serum bilirubin mg/100 ml	Serum phosphatase units	Serum cholesterol mg/100 ml	Total albumen	proteins globulin	A/G ratio	Colloid Gold Reaction	H.A.	G.T.
23	M33	Arseno-therapy		10/5/45 24/5/45 14/6/45 26/6/45	6 8 3 0	+	+	6.0 17.0 3 2	12 22 11 14	161 169 204 177	6.1 5.1 6.5 6.5	1.9 2.1 2.7 2.7	2.2 1.5 1.4 1.4	0 1 0 0	0.2 0.2 0.9 -	72 - - -
24	M29	Arseno-therapy	4	17/9/42 23/6/44 19/6/45	2 0 0	+	- tr 0	0.2 0.5 0.5	10 4 6	- 178 242	- 6.8 7.3	- 2.0 3.1	- 2.4 1.3	- - 0	- 1.05 -	- 33 -
25	F71	Epidemic	5	5/10/42 22/11/43 22/5/45	0 2 4	0 0 0	+	4.4 0.5 0.8	37 - 24	315 - 175	7.6 - 6.9	3.0 - 2.6	1.5 - 1.6	- - 0	- - -	78 55 -
26	M41	Epidemic	8	15/5/45	6	+	+	2.2	11	146	8.1	4.4	0.8	5	0.3	111
27	M22	Epidemic	19	13/11/43	4	0	+	1.5	4	-	7.0	2.3	2.0	-	1.0	33
28	M33	Arseno-therapy	14	27/3/45 10/5/45 7/8/45	2 2 2	++ ++ +	+	0.7 0.5 0.6	11 5 10	162 244 186	6.7 7.5 7.3	2.5 2.7 3.1	1.7 1.8 1.3	0 0 0	0.95 - -	69 - -

In this and succeeding tables

BSP = Bromsulphthalein excretion test percentage retention in serum at 30 minutes

H.A. = Hippuric acid excretion in g. (as sodium benzoate).

G.T. = Galactose time minutes.

hippuric acid and galactose tests, can readily be diagnosed in the laboratory. However this state of affairs is exceptional and cases 23, 24, 25 and 28 now show no biochemical abnormalities with routine methods. The most reliable laboratory methods for the diagnosis of post hepatitis cirrhosis are the technically simplest. They are all related to bile pigment metabolism and are the bromsulphthalein excretion test, the level of the serum bilirubin and the presence of excess urobilin in the urine. If all these are normal then an active cirrhosis is unlikely, a hepatic lesion at a latent stage cannot be eliminated.

Discussion. It seems undoubtedly true that cirrhosis of the liver can follow acute hepatitis. The relation between this lesion and classical Laennec's cirrhosis is uncertain. Most patients with classical cirrhosis will admit to no previous attack of jaundice. Ratnoff & Patek (1942) described the natural history of Laennec's cirrhosis; in only 6.5 per cent of 386 cases was a history of previous jaundice elicited. These figures do not support the contention that acute hepatitis is a major factor in the production of "portal" cirrhosis. However, an original attack of hepatitis without icterus is possible. Hepatitis sine icterus is well known (Jones and Minot, 1923; Damodaran & Hartfall 1944; U.S.Army instruction, 1944; Barker, Capps & Allen, 1945). Moreover, as we have described

in many instances the jaundice is slight and transient and could be unrecognised in patients not in hospital. A fairly severe hepatic lesion has been demonstrated in these cases. It seems possible that in many instances the initial attack may be passed over as an infection and easily forgotten with the passage of years. As Bloomfield (1938) suggests chronic hepatitis may develop in a similar fashion to chronic glomerular nephritis (Bright's Disease). In chronic nephritis a history of an acute attack is often lacking. The present evidence is that acute hepatitis is one, but not the only aetiological factor in the production of Laennec's cirrhosis.

The activity of the lesion in post hepatitis cirrhosis is so variable that the assessment of progress is a matter of extreme difficulty. Case 26 shows clinical, biochemical and histological evidence of activity. Most clinicians would give this patient a poor prognosis. However case 28, at the time of the first observation, seemed nearly as active. 3 months later he is biochemically normal and fit for a full day's work. It seems that, at any time, an active lesion may heal sufficiently for compensation to occur. It is even more difficult to decide the outlook in the histologically inactive cases. The existence of cirrhosis in a latent form is known (Snell, 1931; Bloomfield, 1938). Other authors have reported episodes of "decompensation" many years after an

initial attack of jaundice (Fiessinger, Albot and Thiebaut, 1932). At present we have no definite evidence that the cirrhosis demonstrated in our latent cases is progressive. However the longest period over which they have been observed is 3 years. This is not long enough for a definite pronouncement of permanent healing to be made. The histological picture in some of these cases may represent replacement fibrosis. In the majority of cases of acute hepatitis the reticulin framework is preserved intact. In the very severe lesion the framework can be destroyed and the picture is virtually that of acute or subacute liver necrosis. When the lesion heals it seems possible that residual fibrosis may be present not only in the portal tracts but also through the lobule destroying its essential architecture. The picture is then that of "healed yellow atrophy" (Wilson & Goodpasture, 1927; Mallory, 1923). This lesion is not progressive and provided sufficient liver tissue remains or is regenerated liver function may be adequate.

It is impossible to predict which case of acute hepatitis will progress to cirrhosis. Soffer and Paulson (1934) found no relationships between the severity of the acute attack and the degree of residual hepatitic damage. Six of Polack's eight cases were initially mild with slight icterus lasting only a few days. Krarup & Roholm (1941) suggest that chronic hepatitis should be kept in mind when the course of

hepatitis is prolonged or when the condition shows a tendency for recurrence. On the whole our cases have followed the severer grades of hepatitis. In 4 the jaundice lasted longer than 2 months, in one of these there was a relapse, in another the jaundice was associated with ascites. In two of our cases the acute episode was mild. Cirrhosis can follow acute hepatitis of all grades of severity. Its occurrence after any one type of lesion cannot be predicted.

The results of biochemical investigations of liver function bear a close relation to the histological picture of the liver. Excretion of pigments is often defective and estimations of serum bilirubin and urinary urobilin together with the bromsulphthalein excretion test are the most useful methods for diagnosis. They will not necessarily demonstrate the latent case. It seems that the regenerated and surviving liver cells can provide adequate function as regards carbohydrate and protein metabolism and detoxication but that the gross disturbance of architecture causes distortion of the bile duct system. This is demonstrated by tests involving excretion in the bile. Preliminary work with the cholic acid tolerance test (Josephson, 1939), a test which also seems to reflect small changes in the biliary tree, suggests that this might be useful in diagnosing cirrhosis after hepatitis.

Although residual portal scars are commonly encountered in the liver after clinical recovery from

acute hepatitis a true cirrhosis with disturbance of the lobular architecture and a nodular hyperplasia of liver cells is not commonly recognised. That we have collected six such cases in a series of less than a hundred cases of acute hepatitis is due to the severer cases being hospitalised and also to the selection for biopsy of cases evidencing incomplete recovery. It is suggested however that if a routine aspiration liver biopsy were performed after acute hepatitis before discharge from hospital many other instances might be detected.

CHAPTER 14.

THE POST HEPATITIS SYNDROME

In the last chapter some structural hepatic defects which may follow acute hepatitis were described. A further group of cases have been encountered in which definite symptoms date from a previous attack of hepatitis. In this group neither recognisable biochemical changes or alterations in hepatic histology could be constantly demonstrated. A similar condition following acute hepatitis has been recognised by other workers, (U.S. instruction on Jaundice (1944), Benjamin & Hoyt 1945.) Caravati (1944) has designated this state the "Post-Hepatitis Syndrome".

The clinical features, the biochemical findings and the hepatic histology of a group of these cases will be discussed and some pertinent aetiological factors suggested.

Material. 15 cases were studied. They were all Service men. Cases 1-5 were members of the British Army and cases 6-15 of the Canadian.

Methods. The laboratory methods employed are those described in the previous section.

Results. The observations are summarised in Tables 30, 31 and 32.

TABLE 30

SYMPTOMS AND SIGNS IN POST HEPATITIS SYNDROME

Case	Sex & Age	Months since Hepatitis	Relapses	Fatigue	Weight loss (lb)	Appetite	Nausea & Vomiting	Fat Intolerance & Flatulence	Abdominal discomfort	Dyspnea on exertion	Alcohol intake	Liver Splenomegaly	Urine Urobilin
1.	M. 30	4	0	Yes	14	fair	0	0	0	0	slight	3	tr
2.	M. 31	17	0	Yes	28	poor	occ.	yes	Yes	Yes	slight	0	0
3.	M. 26	15	5	Yes	21	poor	0	0	0	Yes	+	0	0
4.	M. 43	3	0	Yes	8	fair	Yes	Yes	0	0	++	0	0
5.	M. 42	3	2	Yes	0	fair	0	0	Yes	0	0	6	0
6.	M. 21	5	0	Yes	0	fair	occ.	Yes	Yes	0	++	0	0
7.	M. 24	6	0	Yes	21	variable	0	Yes	Yes	0	++	0	tr
8.	M. 26	7	0	Sl.	0	good	0	Yes	0	0	++	7	0
9.	M. 36	6	1	Yes	0	variable	0	0	0	0	slight	4	tr
10.	M. 37	6	1	Yes	14	variable	Yes	0	slight	0	++	4	0
11.	M. 33	7	0	Yes	14	poor	0	0	0	Yes	++	4	0
12.	M. 31	2	2	Yes	14	poor	0	0	0	Yes	++	4	0
13.	M. 23	2	0	0	0	good	0	0	0	0	slight	4	0
14.	M. 23	4	0	Yes	0	variable	occ.	0	Yes	0	++	2	0
15.	M. 29	2	0	Yes	0	variable	0	0	0	Yes	++	6	0

SYMPTOMS.

Fatigue. This caused the most disability. It occurred in 13 of the 15 cases. All had been grade A.1. before the acute hepatitis. Now they complained of lack of energy and "pep". They were exhausted with minimal exertion. Some had lost all ambition for their post-war careers.

Weight loss. Eight cases had failed to regain the weight lost during the acute episode of hepatitis. They were between 8 lbs. and 2 stone below their usual weights.

Dyspnoea on exertion. 5 cases complained of breathlessness on exertion. It was usually associated with fluttering and palpitation in the chest. There was no instance of organic heart disease.

Gastro intestinal Symptoms. Poor and variable appetite was encountered in 7 cases. In only 4 was there a definite aversion to fatty foods. Most of the men were "faddy" about their food and some had strange dislikes, one would never eat bread, another could not take meat. Flatulence was a feature in 5 cases. 4 complained of occasional nausea and vomiting.

Some complained of right upper abdominal discomfort. It was an unpleasant ache rather than an acute pain. It was usually brought on by exercise and especially lifting. It was relieved with rest in bed.

Psychiatric Make up. A detailed psychiatric study of each case was not attempted. Certain points however were ascertained.

Of the British group 3 were psychologically imbalanced. One, an officer, was extremely introspective.

A man of independent means, he had consulted many London Physicians of repute before arriving at the Postgraduate School. Another had troubles at home, he had been a deserter for 3 months and was eventually caught in a semistarved state and covered with a pustulent syphilitic eruption. The third was a very nervous young man, also introspective, who had just been invalided out of the Army with "Effort Syndrome".

The Canadian group were on the whole well balanced. They however had all been hospitalised for at least 3 months. They were all treated in the same ward. The medical officer in charge was very conscientious and the liver was regularly examined and records made of its exact size. Moreover the men were in hospital at the end of European Hostilities when there was some delay in repatriating troops to Canada. It was believed that sick men would receive priority passages. A few had matrimonial entanglements both here and in Canada.

Other Diseases. Most of the men had served in the African or Italian theatres. One man (case 3) had had 3 attacks of malaria. 3 had suffered from bacillary and one from amoebic dysentery. All these conditions had been adequately treated.

Relapses of Hepatitis. Five gave histories of more than one attack of hepatitis. One case, (No.3) was said to have had 5 episodes. This led to fear of further attacks and of permanent damage to the liver.

Alcohol. 9 men admitted to excessive consumption of alcohol. 3 took only moderate amounts and 3 were almost t.t. Many of the Canadians had been drinking fairly heavily prior to admission and while in Hammersmith Hospital were given afternoon and evening passes. During this time, in spite of medical advice to the contrary, they freely refreshed themselves.

Examination.

General development. Although a large proportion complained of being underweight the general impression of the group was of adequate nutrition.

Hepatomegaly. In 13 of the cases the liver was palpable. The size was between 2-7 cm. below the right costal margin in the nipple line. The measurement was made in inspiration. The liver edge was smooth and rubbery in consistence, it was not usually unduly firm. Tenderness was not elicited.

Splenomegaly. In one case of the fifteen the spleen was just tipped.

Urine Examination. 3 cases showed a constant slight excess of urobilin in the early morning specimen.

Laboratory findings.

TABLE 31.

TABLE 31

LABORATORY FINDINGS IN THE POST HEPATITIS SYNDROME
Biochemical Findings

Case No.	Serum bilirubin mg/100 ml	Serum phosphatase units 100ml	Serum cholesterol mg/100 ml.	Serum Protein g/100 ml			Coll-oidal Gold	B.S.P.	H.A.	G.T.
				Total	Albumen	Globulin				
1.	0.5	8.3	198	6.8	4.6	2.2	2.1	0	0.7	72
2.	0.5	3.2	183	6.6	4.4	2.2	2.0	0	1.1	33
3.	0.5	7.0	187	7.0	5.0	2.0	2.5	0	0.99	32
4.	1.0	14	-	6.5	3.7	2.8	1.3	-	0.84	-
5.	0.5	10	183	6.7	4.3	2.4	1.8	-	0.9	-
6.	1.3	6	318	6.9	3.7	3.2	1.1	0	1.1	-
7.	0.9	6	199	6.8	3.1	2.7	1.1	0	0.95	-
8.	0.5	7	174	7.4	5.1	2.3	2.2	0	1.0	-
9.	1.2	2	298	7.3	4.0	3.3	1.2	5	0.7	-
10.	0.6	5	236	6.2	3.4	2.8	1.2	3	0.98	-
11.	0.6	2	220	7.0	4.0	3.0	1.3	1	1.0	-
12.	0.5	6	309	6.3	3.5	2.8	1.2	2	0.9	-
13.	0.5	6	229	7.5	4.0	3.5	1.1	1	1.0	-
14.	0.5	3	148	6.8	3.7	3.1	1.2	1	1.1	-
15.	0.5	4	270	6.0	3.4	2.6	1.3	6	1.2	-

Serum Bilirubin. In three cases (numbers 4, 6, and 9) the serum bilirubin was at the upper limit of normal. The others were normal.

Serum Phosphatase. In only one case was the serum phosphatase greater than 10 units per 100ml. This man was observed only 3 months after the original attack of hepatitis. A raised serum phosphatase is not unusual for some time even in apparently normal recovering cases of acute hepatitis.

Serum Cholesterol. 5 cases showed serum cholesterol values above the upper limit of normal (230mg/100ml). In 2 cases it was greater than 300 mg/100ml. The significance of this finding will be discussed later.

Serum Proteins. The group showed no significant abnormalities from normal values in the total serum protein, the serum albumen, serum globulin or A/G ratio.

Colloidal gold reaction. Of 13 tests two gave a weakly positive reaction.

Bromsulphthalein excretion test. Normal in all the cases studied.

Intravenous hippuric acid test. 2 cases gave a low value. In the other 13 tests the excretions were normal.

Intravenous Galactose Tolerance Test. It has been shown previously that the galactose test is of little value in the study of liver disease without jaundice. It was performed in only 3 of this series. All were normal.

Liver Biopsy Findings.

TABLE 32.

TABLE 32

HEPATIC HISTOLOGY IN THE POST HEPATITIS SYNDROME

Case No.	Hepatic cells & lobular pattern	Portal tracts	Glycogen	Fat	Iron	Kupffer cells
1.	normal	normal	normal	absent	absent	normal
2.	normal	normal	normal	absent	absent	normal
3.	normal	normal	normal	absent	absent	normal
4.	normal	sl. excess fibrous tissue	normal	absent	absent	normal
5.	normal	"	normal	slight excess	absent	normal
6.	normal	normal	slightly deficient	"	absent	normal
7.	normal	normal	normal	absent	absent	normal
8.	normal	normal	normal	absent	absent	normal
9.	normal	normal	patchy	slight excess	absent	normal
10.	normal	normal	slightly deficient	"	absent	normal
11.	normal	normal	normal	"	absent	normal
12.	normal	sl. excess fibrous tissue	normal	slight at periphery	absent	normal
13.	normal	normal	normal	slight	slight excess	slight excess
14.	normal	normal	normal	absent	absent	normal
15.	normal	normal	normal	sl. excess	absent	normal

Hepatic cells and lobular pattern. The cells appear normal. The lobular pattern is not disturbed. There is no evidence of continuing hepatitis or of cirrhosis.

Portal tracts. Three cases, all within 3 months of recovery from the initial attack of jaundice, show slight excess fibrous tissue in the portal tracts. The scarring is relatively acellular, bile duct proliferation is not noted. The picture resembles that of residual portal scarring described previously.

Glycogen content of the liver. The glycogen content of the liver cells is on the whole well preserved. In two instances the glycogen is slightly deficient and in another there is marked patchiness of glycogen, small areas containing a good complement, others containing little or none. The glycogen deficiency bears no particular position in the liver lobule.

Fatty change in the liver. Slight excess of fat is present in 8 instances. This fatty change is the only abnormality encountered in the liver cells with any frequency. The fat stains well with osmic acid. It usually takes the form of one or two droplets in the liver cells. Distension of the liver cells with fat so that the nucleus is pushed to one side is not observed. In general the fat is evenly distributed through the lobule. In one instance (case 12) it is largely found at the periphery of the lobules.

Iron. is absent both from the Kupffer cells and from the liver cells. In one case only (No. 13) is there slight excess in both situations.

Kupffer cells. One case shows slight excess. The others are within normal limits.

Discussion. Very large numbers of men in the forces have contracted acute hepatitis. Most of them make an excellent recovery. A small proportion complain of persistent symptoms after apparent clinical cure. Already Benjamin & Hoyt (1945) have described a group of 200 cases occurring in United States troops after yellow fever serum jaundice. Caravati (1944) has published a similar smaller series. It is essential to recognise the true organic sequelae from those in which symptoms are based on a less firm structural basis. Moreover it is apparent that the symptoms of the "post-hepatitis syndrome" are very similar to those occurring in men with definite active post-hepatitis cirrhosis. Some of them present hepatomegaly. A very full examination including biochemical and hepatic histological studies are essential to differentiate the one group from the other. It can be at once categorically stated that the symptoms these men present are out of all proportion to the biochemical or hepatic histological findings.

In the liver the only unusual finding has been about 50% of cases with a slight degree of fatty change in the liver cells. Although fatty change is a common occurrence in sections of liver obtained post-mortem it is very unusual in aspiration biopsy material. In the normal livers we have sampled it has been a very rare finding. Iversen and Krarup (1940), using the

aspiration biopsy technique, in 100 successive punctures collected 38 cases of steatosis hepatis. Of these 27 occurred in confirmed alcoholics. It seems that the fatty change observed in our cases might also be related to alcoholicism. Of the 8 cases who presented fatty change 7 were heavy drinkers. As "controls" aspiration biopsies were done on 3 other Canadian soldiers who presented hepatomegaly but had not had previous hepatitis. All were heavy consumers of liquor. Two showed fatty change in the liver. The raised serum cholesterol was invariably found in the cases whose livers showed fatty change. It may also be related to acute or chronic alcoholicism (Epstein and Greenspan 1936). The patients with the highest serum bilirubin levels had normal bromsulphthalein excretion tests, the bilirubin reading in these subjects is probably a high normal and without pathological significance.

The hepatomegaly may be related to the increased fat content of the liver. Powerful practised diaphragmatic movements may play a part by making the organ more easily palpable.

It might be postulated that the fatty change is a precirrhotic manifestation. This does not seem likely. Cases of acute hepatitis proceeding to cirrhosis have not shown noteworthy fatty change in the liver. Fatty change has not been conspicuous in the group in which Laennec's cirrhosis arises apparently "de novo". With Iversen and Krarup (1940), we prefer to believe that the majority of instances of cirrhosis originate on the basis of inflammation rather than steatosis.

Although a detailed psychiatric study was not undertaken, certain points did emerge. We have yet to observe this syndrome in civilians. Many of the men were considering their pension rights. The syndrome seems commoner in troops overseas than in home forces. Some feel the disease may be an opportunity for repatriation. Acute hepatitis is nearly always an unpleasant disease. It usually lasts a minimum of 3-4 weeks. Convalescence is slow. If the illness relapses, as it did in many of this group, fear may arise of further attacks and of permanent liver damage. This fear is accentuated when a number of patients with the same complaint are warded together. Two or three men would often give the same complaints in almost identical terms. All the patients volunteered for liver biopsy. Many of them derived great benefit from the reassurance possible after examination of hepatic sections. The treatment, as in "effort syndrome" which it rather resembles, must be reassurance and rehabilitation after the fullest possible investigation.

CHAPTER 15.

ACUTE HEPATITIS

Summary of chapters 11-14

The general pathology of 87 cases of acute hepatitis has been studied by aspiration biopsy.

The inflammatory lesions may be diffuse, zonal, or mixed. Jaundice persisting over 2 weeks is more likely to be due to a zonal lesion.

No evidence has been found that there is a form of jaundice due to duodenal catarrh and obstruction of the common bile duct by mucus.

No histological criteria have been found for the differentiation of the lesions resulting from epidemic hepatitis, arsenotherapy and serum inoculations.

Fatal acute hepatitis is rare. The histological picture in the liver is that of acute or subacute liver necrosis depending on the duration of illness.

The recovery process has been studied by serial biopsies in 11 cases. Recovery is usually rapidly completed. The basis for a regular regeneration of liver cell columns is an intact reticulin framework. Portal zonal scarring may remain after complete recovery elsewhere. It appears to be of no clinical significance.

Biochemical changes as recovery ensues have been stated and discussed.

A small proportion of cases of acute hepatitis pass to permanent hepatic structural alterations. The

lesion is a hepatic cirrhosis. Six such cases are described. The degree of activity in each is variable. The prognosis is uncertain.

A further group of cases have persisting symptoms after hepatitis. There is no adequate explanation of the complaints, either in the biochemical findings or in the hepatic histology. The group have been designated the "post-hepatitis syndrome". Aetiological factors in the production of the syndrome are discussed.